QUANTIFYING THE IMPACT OF AN URBAN ONSITE SHARED SANITATION INTERVENTION ON CHILD HEALTH IN MAPUTO, MOZAMBIQUE: THE MAPSAN TRIAL

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QUANTIFYING THE IMPACT OF AN URBAN ONSITE SHARED SANITATION INTERVENTION ON CHILD HEALTH IN MAPUTO, MOZAMBIQUE: THE MAPSAN TRIAL

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Dedication

To MapSan Trent and regular Trent. I could not have done this without either of you

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LIST OF SYMBOLS AND ABBREVIATIONS

alpha-1-anti-trypsin	AAT
adjusted risk ratio	aRR
calprotectin	CAL
Celsius	C
communal sanitation block	CSB
confidence interval	CI
controlled before-and-after	CBA
difference-in-difference	DID
degree	°
double-stranded deoxyribonucleic acid	dsDNA
enterotoxigenic *E. coli*	ETEC
environmental enteric dysfunction	EED
enzyme-linked immunosorbent assays	ELISA
Escherichia coli	*E. coli*
Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development	MAL-ED
fecal indicator bacteria	FIB
Gastrointestinal Pathogen Panel	GPP
generalized estimating equations	GEE

Global Enteric Multicenter Study	GEMS
health impact evaluation	HIE
lower and middle income countries	LMICs
Maputo Sanitation	MapSan
microliter	µL
milligrams	mg
milliliter	mL
moderate to severe diarrhea	MSD
multiple imputation	MI
myeloperoxidase	MPO
neopterin	NEO
registered trademark	®
ribonucleic acid	RNA
risk ratio	RR
rotations per minute	rpm
shared latrine	SL
shiga-like toxin producing *E. coli*	STEC
Sanitation Hygiene Infant Nutrition Efficacy [study]	SHINE
soil-transmitted helminth	STH
Trademark	™
Water and Sanitation for the Urban Poor	WSUP
water, sanitation, and hygiene	WASH
Water, Sanitation, and Hygiene Benefits [study]	WASH-

	B
x gravity	x g

SUMMARY

Globally, over 50% of the world's urban population, or approximately 2.2 billion people, lack access to safely managed sanitation (1). Inequities in access increase in low-resource regions like Sub-Saharan Africa, where over 80% of urban residents lack access to safely managed sanitation (1). Because development of advanced wastewater infrastructure may be decades away for rapidly growing cities of developing nations, there is increasing interest in decentralized sanitation technologies that serve urban clusters. Though various methods for improved on-site urban sanitation have been piloted, the impacts of such strategies on reducing exposure to enteric (gut) pathogens and subsequent effects on enteric infections have not been characterized. Here we present results from the Maputo Sanitation (MapSan) trial, the largest controlled health impact study to date of decentralized urban sanitation in the developing world (2). In this study, we examined the expansion of improved on-site sanitation serving household clusters (compounds) in low-income, densely populated urban neighborhoods of Maputo, Mozambique, where the burden of sanitation-related disease is high. We measured objective health outcomes in children <6 years old before and after the implementation of new sanitation systems which included pour-flush toilets to septic tanks with soak-away pits to discharge aqueous effluent. In parallel, we measured the same outcomes in children enrolled in control compounds that did not receive the new latrines. We used a difference-in-difference (DID) analytical approach, coupled with our controlled before-and-after study design, to estimate the effects of the intervention on three metrics of child health: enteric

infection, environmental enteric dysfunction (EED), and reported diarrhea. We collected stool from enrolled children at three time-points during the study: pre-intervention (n=757), 12-months post intervention (n=803), and 24-months post-intervention (n=923). We tested stools for enteric pathogens using a qualitative multiplex molecular method detecting 15 enteric bacteria, protozoa, and viruses identified as important causative agents of diarrhea and four biomarkers of EED using enzyme-linked immunosorbent assays.

Enteric infection was common at baseline with over 80% of children in both intervention and control arms positive for one or more pathogen. The sanitation improvements had no effect on enteric infections when we analyzed results from the 12-month and 24-month time-points separately or combined into a single "follow-up" phase. We also found no effect on caregiver-reported diarrhea over the course of the study. Sanitation increased the concentration of EED biomarker neopterin, an indicator of immune system activation and inflammation, by 0.17 $\log_{10}$ nmol/L (95% CI: 0.07 - 0.27) at the 12-month time-point and 0.10 $\log_{10}$ nmol/L (95% CI: -0.01, 0.21) at 24-months. Results from sub-group analyses of children with data collected at multiple time-points and children born into the study post-intervention were largely similar to the main analyses. We observed a reduction in risk of *Shigella* infection by almost 50% (adjusted risk ratio: 0.53, 95% CI: 0.29-0.95) in children born into intervention compounds by the 24-month time-point. There are many potential reasons the intervention had a limited effect on child health in this setting. It may not have interrupted all transmission pathways of import or limited fecal contamination of the living environment to the extent necessary to observe real

changes in exposure. In these densely populated, low-income urban areas, sources of contamination are ubiquitous and our intervention sites may have been impacted by poor water, sanitation, and hygiene (WASH) conditions in surrounding areas. Delivery of comprehensive WASH interventions at a community level may be necessary to realize real health gains in similar settings.

INTRODUCTION

Clean water and safe sanitation have been recognized as basic human rights that are necessary to the achievement of all other human rights. Unfortunately, these international normative goals are only partly realized, and over 2 billion people live without access to basic sanitation services and 785 million lack access to basic water services (1). This burden is not equally distributed and falls largely on the disenfranchised, poor, and most vulnerable among us. In Sub-Saharan Africa, only 44% of urban residents have access to basic sanitation (improved sanitation infrastructure), leaving approximately 228 million people without basic services and an additional 98 million without access to safely managed sanitation (improved sanitation infrastructure plus safe disposal and treatment of waste) (1). While these numbers represent progress in increasing access generally, progress is not equitable and in many places often favors the richest. In Mozambique, where cities are quickly expanding, the proportion of the urban population without access to basic services decreased from 68% in 2000 to 48% in 2017, however, gains among the richest quintile of the population far outpaced progress among the poorest, increasing the gap in access between them by 30 percentage points (1). Population growth and increasing migration to cities will put additional pressure on already fragile urban sanitation systems in many places (3–5). In fact, due to population growth in Sub-Saharan Africa, the total population using unimproved facilities increased by almost 140 million between 2000 and 2017, the only developing region to experience an increase in use of unimproved sanitation without a corresponding decrease in open defecation (1).

The primary goal of safe sanitation systems is to sequester human waste away from human contact. Use of failing or inadequate sanitation infrastructure can lead to fecal contamination of the environment increasing the risk of human exposure to enteric (fecal) pathogens (6–11). Enteric pathogens are transmitted via several fecal-oral pathways historically defined by the F-diagram (Figure 1) (11). Consumption of contaminated food and water and interaction with fecally contaminated environments have been implicated as dominant transmission pathways for bacterial and protozoan enteric pathogens (12,13). While enteric viruses can be transmitted via similar environmentally-mediated routes, it is posited that person-to-person transmission is also important (14). Exposure to enteric pathogens via unsafe water, sanitation, and hygiene conditions (WASH) can result in in enteric infections and acute and chronic diarrheal disease, and has been associated with environmental enteric dysfunction (a disorder affecting the function and structure of the gut), malnutrition (poor growth), cognitive deficiencies, and other long term health and well-being outcomes (6,15–23). In 2016, unsafe water and sanitation contributed to 72% and 56% of diarrheal deaths in children <5 years old, respectively (16).

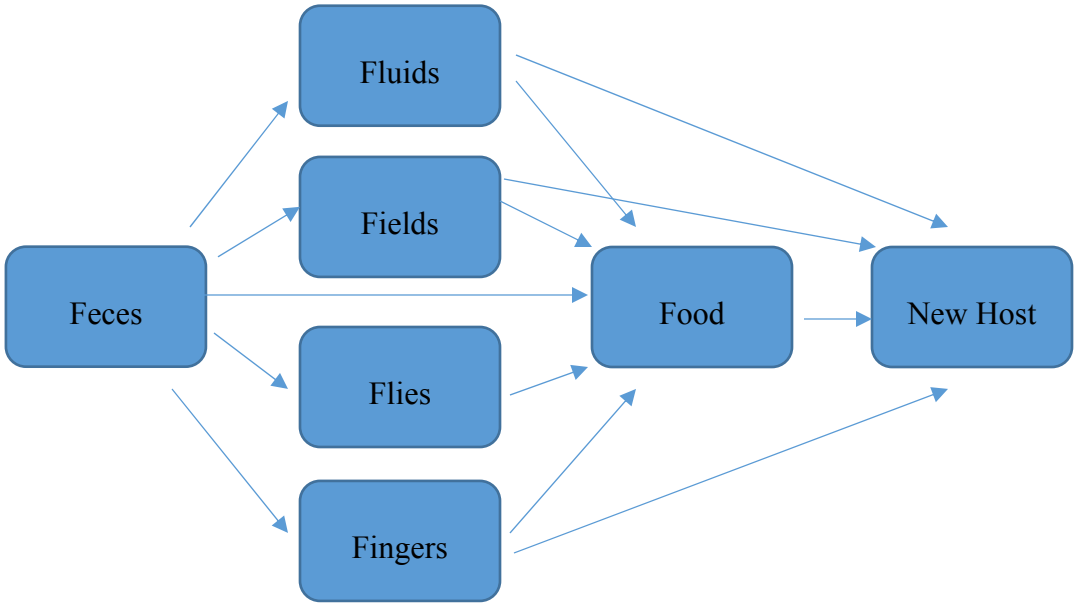

FIGURE 1: The F-diagram describes major environmentally-mediated routes of transmission of enteric pathogens. Adapted from Wagner and Lanois 1958 (11).

Urban slums - or low-income, unplanned neighborhoods in urban or peri-urban areas - represent high risk settings for enteric pathogens transmission, particularly among vulnerable populations like children, as they are characterized, in part, by high population density and crowding, poverty, limited access to adequate water and sanitation services, and high infection burdens (3,5,24,25). Developing effective WASH interventions to interrupt transmission of enteric pathogens is a necessary, albeit complex, step to protect public health in these settings. Traditional centralized sewerage systems, often used in urban settings, have demonstrated health benefits both historically and in several recent evaluations of sewerage expansion projects in Brazil and Iran (26–28). While these types of systems are aspirational, their implementation may not be feasible in the near term due

to cost and logistical constraints (26,29). Sanitation solutions for urban slums will require technologies and service models that can deliver benefits at lower cost.

On-site systems such as septic tanks with soakaway pits or small-bore sewers may fill the growing need for safe sanitation in rapidly expanding urban areas in low and middle income countries (LMIC) (26,30). To date, there is limited evidence of the health impact such systems may have in these settings. A recent meta-analysis found that sanitation interventions reduce the risk of diarrhea by 25%, though the estimate was reduced to 16% when limited to non-sewerage interventions (31). Recent large-scale, rigorous trials of on-site sanitation and combined WASH interventions in low-resource areas have found mixed evidence of impact on human health (32–36), but all were performed in rural areas and their findings may not be generalizable to urban settings. Two trials in India found no effect on health outcomes such as self-reported diarrhea, growth, protozoan infection or soil-transmitted helminth (STH) infection, likely due to poor uptake and sustained use of the intervention (34,35). In contrast, a study of a community-led total sanitation (CLTS) intervention in rural Mali observed better growth outcomes in children in the intervention study arm but no effect on diarrhea (36). The Water, Sanitation, and Hygiene Benefits (WASH-B) trial and the Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial, both cluster-randomized trials studying the effects of WASH and nutrition interventions on child health in LMICs, found modest effects of nutritional interventions, but not WASH, on child growth (32,33,37). The WASH-B Bangladesh site found reductions in diarrhea due to the intervention (from 5.7% to 3.5% prevalence) (33), but WASH-B Kenya site and SHINE trial in Zimbabwe did not (32,37). Recently, many of these trials have

released findings on their secondary outcome measures such as enteric infections and the results are similarly mixed. In Bangladesh, the on-site sanitation intervention reduced the prevalence of *Giardia* (25% reduction) and *T. trichiura* (29% reduction) (38,39), and in Kenya the combined WASH intervention, but not the individual sanitation intervention, reduced the prevalence of *Ascaris* but not *Giardia* or *T. trichiura,* the latter of which was detected in too few samples to precisely estimate (40). In the Zimbabwe trial, the combined WASH intervention decreased the number of parasites detected but did not reduce the prevalence of any single pathogen. The combination of a WASH and nutrition interventions resulted in an absolute reduction in *Giardia* prevalence by 10% (41). Evidence from these trials is mixed for several potential reasons: (1) a lack of acceptability, sustainability, and use of the intervention, (2) failure of the intervention(s) to adequately limit exposure to fecal contamination (possibly due to inadequate coverage levels), (3) selection of inappropriate study outcomes due to flaws in hypothesized causal pathways, (4) the abbreviated length of follow-up periods, and (5) inter-site differences in behaviors, exposures, and circulating enteric pathogens (42).

Health impact of enteric infection

Diarrheal disease, a commonly used metric of health in WASH intervention trials, is largely caused by enteric pathogens, including bacteria, viruses, and protozoa, shed in human and animal feces. Until recently, the main etiologic agents of childhood diarrhea in LMICs were poorly characterized. The Global Enteric Multicenter Study (GEMS), a large-scale study of the etiology of moderate to severe diarrhea (MSD) in seven sites located in LMICs in Africa and Asia, found that the majority of MSD in children less

than five years of age was caused by just four of the 17 enteric pathogens measured: rotavirus, *Cryptosporidium* spp., enterotoxigenic *Escherichia coli* (ETEC), and *Shigella* spp (43). The contribution of these pathogens and others to the burden of MSD varied with age and site location. For example, rotavirus had the highest attributable fraction of MSD in infants aged <12 months but its relative importance diminished with age across most sites (43). Conversely, the proportion of MSD attributed to *Shigella* tended to increase with age. Some pathogens were regionally important, suggesting specificity of diarrheal etiology to setting: in the Mozambique site, adenovirus was a frequent etiologic agent of MSD whereas *Aeromonas* was important in the Bangladesh and Pakistan sites (43,44). Another large scale multicenter study, the Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study, identified causative agents of any diarrhea, mild, moderate, or severe, (measured as caregiver reported diarrhea) in children less two years of age across 8 sites in LMICs in South America, Africa, and South Asia (45). Results from the MAL-ED study found that norovirus GII, rotavirus, astrovirus, *Campylobacter*, *Shigella*, and *Cryptosporidium* were the most frequent causative agents of diarrhea across study sites (46). Again, the importance of specific pathogens varied with age and geography as well as other factors such as deployment of the rotavirus vaccine in a given region.

Enteric pathogens can be shed in high numbers by both symptomatic and asymptomatic individuals (43). In GEMS, one or more enteric pathogen was detected in 83% of children with MSD and 72% of matched control children (children without diarrhea)

(43). In the MAL-ED study, one or more pathogen was detected in 77% of diarrheal stools and 65% of non-diarrheal surveillance stools (46). Although the immediate and longer-term health and productivity effects for asymptomatic individuals are unclear (47), persistent asymptomatic infections are associated with environmental enteric dysfunction (21,23,48). Environmental enteric dysfunction (EED) is a subclinical and poorly defined condition affecting the function and structure of the gut. At present, no formal case definition for EED exists (49), though EED is often characterized by the flattening or blunting of the intestinal villi, malabsorption, increased intestinal permeability, and aberrations in immune system activation (18,22,50–53). EED, originally described as tropical enteropathy, was first identified in the mid-twentieth century in local populations and expatriate populations living in tropical regions where sanitary conditions tended to be poor (54). Upon repatriation or migration to an industrialized nation, the pathophysiologies associated with EED, such as reduced villus height, typically resolved within weeks to years (55–57). These initial studies of EED were the first to suggest that environmental exposures may drive the onset of or recovery from EED. While the precise etiology of EED is unknown, it is postulated that it develops in response to repeated exposure to fecal contamination and enteric pathogens - common exposures in low-resource settings where sanitary conditions can be poor (21,22,58).

When first identified, EED was often compared with the condition known as tropical sprue: a disorder which exhibits many of the same pathophysiological changes as EED including flattening of the intestinal villi, nutrient malabsorption, and immune activation leading to diarrhea, weight loss, fatigue, fatty stool, and signs of nutrient deficiencies.

Patients with tropical sprue often recover after receiving courses of folic acid supplementation and antibiotics. Unlike tropical sprue, EED tends to be subclinical with affected persons displaying no overt symptoms, and typical treatments for tropical sprue tend to have no effect on the course and persistence of EED. The effects of EED, like gut inflammation, malabsorption, and intestinal permeability are believed to contribute to malnutrition which together with the other manifestations of EED can lead to increased susceptibility to future infections, completing the cycle of infection, EED, and malnutrition (23)pred. The onset and persistence of EED, especially at a young age, has been empirically linked to poor growth outcomes (22,58–60), though the precise mechanism through which EED contributes to stunting is unclear (22). Enteric infection and EED have also been linked to other serious or long-term health and well-being effects including reduced immunogenicity of oral vaccines (52,61,62) and cognitive deficits (63–65).

The lack of a precise case definition makes measurement of EED difficult. While the optimal method for assessing EED is measurement of villus height and morphology and crypt depth in a biopsied section of the intestinal mucosa or via endoscopy, this method is highly invasive - especially in children with no overt symptoms - and can present technical and logistical difficulties. Numerous biomarkers that can be assessed in stool, urine, and serum represent less invasive alternatives to traditional microscopic evaluation of biopsied specimens. Several pathophysiological parameters have been proposed as defining characteristics of EED: intestinal (local) inflammation, systemic inflammation, intestinal permeability and malabsorption, microbial translocation, and intestinal damage

and repair, though there is overlap among these domains and the biomarkers used to measure them (22,50,51). A brief overview of biomarkers frequently used to assess EED is presented in Table 1.

Table 1: Biomarkers of the five domains of EED and specimen type required for measurement (22).

EED Domain	Biomarkers	Specimen type
Intestinal inflammation	Myeloperoxidase, neopterin, calprotectin, "EED score"[1]	stool
Systemic inflammation	total IgG and IgM, α-1-acid glycoprotein, c-reactive protein, ferritin, soluble CD14, interferon gamma, tumour necrosis factor, interluekins	serum
Intestinal permeability/malabsorption	Lactulose-mannitol ratio α-1-anti-trypsin	urine stool
Microbial translocation	Elevated endotoxin core antibody, anti-lipopolysaccharide IgG and IgA	serum
Intestinal damage & repair	Citrulline, regenerating proteins, Glucagon-like peptide 2, intestinal fatty acid binding protein	serum

[1] "EED score" is a weighted combination of results from assays of myeloperoxidase, neopterin, and alpha-1-anti-trypsin developed by Kosek et al. (59).

Measurement of fecal biomarkers of EED represents one of the least invasive methods of assessing EED and several biomarkers have been proposed and used for this purpose including alpha-1-anti-trypsin (AAT), myeloperoxidase (MPO), neopterin (NEO), and calprotectin (CAL). NEO and MPO are both enzymes released during an inflammatory response in the gut. CAL is released during intestinal cell damage and is considered a marker of inflammation, however, there is some evidence that high CAL concentrations

are correlated with breastfeeding in otherwise healthy infants (66). AAT is also released in response to inflammation, but because AAT is synthesized in blood it is largely considered a marker of intestinal permeability (22). A composite metric of three of these biomarkers (AAT, NEO, and MPO), known as "EED score," was previously developed by Kosek et al. and may better explain linear growth deficits in young children than measurement of any single biomarker (59).

While many biomarkers have been proposed to measure different domains of EED, there is little consensus in the literature regarding which domains and biomarkers best define EED and most strongly associate with potential downstream outcomes like stunting. A systematic review of recent EED literature found either no evidence or conflicting evidence of the connections between most EED domains and stunting (22). The strongest evidence supported the pathway between intestinal inflammation and stunting. The lack of evidence, or conflicting evidence, for associations between the other domains and stunting could be due to a true lack of relationship or to issues with the biomarkers (and methodology) selected to represent those domains.

Health Impact Evaluations

Health impact evaluations (HIE) of WASH interventions, like WASH-B, SHINE and others described previously, serve several purposes. Theoretically, a HIE that aims to have good external validity could demonstrate the expected effect of an intervention on the health and well-being of a population generally. Practically, the generalizability of results from HIEs are limited by several factors related to the study population, setting, and specific intervention (67). Conclusions from HIEs can also aid in the refinement and

revision of hypotheses and causal pathways – leading to more robust future studies. Further, and arguably most importantly, when properly executed HIE can estimate the magnitude of health gains to be expected following implementation of a specific intervention in a specific setting. This context-specific information facilitates comparisons of different WASH solutions for particular locations and helps inform local health and WASH-related policy decisions made by government officials, non-profits, and other WASH practitioners (68).

The Maputo Sanitation (MapSan) trial is a three-year independent HIE of an on-site, privately shared sanitation intervention implemented in low-income, unplanned neighborhoods of Maputo, Mozambique. The interventions we evaluated for this trial were built by the non-governmental organization Water and Sanitation for the Urban Poor (WSUP) which has been building similar latrines in Maputo for over a decade. These sanitation improvements were designed and delivered at the compound level. Compounds, in this context, are clusters of two or more households that share sanitation and outdoor living space and are typically surrounded by a physical barrier (wall or fence) to delineate the compound boundaries. While the intervention is officially considered shared sanitation, as it was meant to be used by two or more related or unrelated households, it not considered public sanitation and was only accessible by members of the compound ("privately shared"). The basic intervention infrastructure consisted of pour-flush latrines (ceramic squat plates or pedestals) to septic tanks with soakaway pits to discharge liquid effluent (Appendix A, Figure A1). Septic tanks were designed to contain approximately two years of waste and included access ports to

facilitate formal emptying services. WSUP constructed different latrine designs depending on the population of the compound but the basic sanitation technology remained the same in both. Communal sanitation blocks (CSBs) were built in compounds with more than 20 members and included multiple latrine stalls (one for every 20 compound members), covered vent pipes for fly control, secure doors with padlocks, a municipal water supply connected to an elevated water storage tank (to allow for semi-continuous water access), a rainwater harvesting system and storage basin with floor level taps, a sink that could be connected to the water supply (connection not performed by WSUP), and a laundry facility (Appendix A, Figure A2). Shared latrines (SLs) were constructed in compounds with 20 or fewer members and included a single latrine stall, a covered vent pipe, and a secure door with a padlock (Appendix A, Figure A3).

MapSan is the first large-scale HIE of a decentralized urban sanitation intervention, the first to study a shared sanitation intervention, and the first to use enteric infection, an objective metric of health, as the primary outcome (2). My work aims to characterize the baseline health status of the study population prior to the introduction of the intervention, understand the main environmental and sanitary risk factors for infection (Chapter 2), and evaluate the impact of the intervention on two metrics of child health: enteric infection (Chapter 3) and environmental enteric dysfunction (Chapter 4).

CHAPTER 2

RISK FACTORS FOR CHILDHOOD ENTERIC INFECTION IN URBAN MAPUTO, MOZAMBIQUE: A CROSS-SECTIONAL STUDY

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ABSTRACT

Background

Enteric infections are common where public health infrastructure is lacking. This study assesses risk factors for a range of enteric infections among children living in low-income, unplanned communities of urban Maputo, Mozambique.

Methods & Findings

We conducted a cross-sectional survey in 17 neighborhoods of Maputo to assess the prevalence of reported diarrheal illness and laboratory-confirmed enteric infections in children. We collected stool from children aged 1–48 months, independent of reported symptoms, for molecular detection of 15 common enteric pathogens by multiplex RT-PCR. We also collected survey and observational data related to water, sanitation, and

hygiene (WASH) characteristics; other environmental factors; and social, economic, and demographic covariates.

We analyzed stool from 759 children living in 425 household clusters (compounds) representing a range of environmental conditions. We detected ≥1 enteric pathogens in stool from most children (86%, 95% confidence interval (CI): 84-89%) though diarrheal symptoms were only reported for 16% (95% CI: 13-19%) of children with enteric infections and 13% (95% CI: 11-15%) of all children. Prevalence of any enteric infection was positively associated with age and ranged from 71% (95% CI: 64-77%) in children 1-11 months to 96% (95% CI: 93-98%) in children 24-48 months. We found poor sanitary conditions, such as presence of feces or soiled diapers around the compound, to be associated with higher risk of protozoan infections. Certain latrine features, including drop-hole covers and latrine walls, and presence of a water tap on the compound grounds were associated with a lower risk of bacterial and protozoan infections. Any breastfeeding was also associated with reduced risk of infection.

Conclusions

We found a high prevalence of enteric infections, primarily among children without diarrhea, and weak associations between bacterial and protozoan infections and environmental risk factors including WASH. Findings suggest that environmental health interventions to limit infections would need to be transformative given the high prevalence of enteric pathogen shedding and poor sanitary conditions observed.

INTRODUCTION

Diarrheal illness is estimated to cause approximately 1.7 million deaths annually and result in over 74 million disability-adjusted life years lost (16), primarily among children in low-and middle-income countries where fecal contamination of the living environment is common. Diarrheal diseases are mostly caused by enteric pathogens, including bacteria, viruses, and protozoa, shed in human and animal feces. These pathogens can be shed in high numbers by both symptomatic and asymptomatic individuals (43). Although the immediate and longer term health and productivity effects for asymptomatic individuals are unclear (47), persistent asymptomatic infections are associated with environmental enteric dysfunction (21,48,69) and other conditions, including undernutrition, poor linear growth, reduced immunogenicity of oral vaccines and cognitive deficits.

Enteric pathogens are transmitted via several fecal-oral pathways historically defined by the F-diagram (11). Consumption of contaminated food and water and interaction with fecally contaminated environments have been implicated as dominant transmission pathways for bacterial and protozoan enteric pathogens (12,13). While enteric viruses can be transmitted via similar routes, it is posited that person-to-person transmission is also important (14). Improvements in WASH conditions can reduce risk of diarrheal disease by interrupting transmission pathways. A recent meta-analysis observed reductions in diarrheal disease risk by an average of 67%, 25%, and 30% for water, sanitation, and hygiene interventions, respectively (31). Sanitation interventions may be more likely to

interrupt transmission of protozoa, bacteria, and helminths which are primarily spread via indirect, environmentally mediated pathways than viruses which are often spread via person-to-person transmission (9).

Densely populated, urban, unplanned communities with inadequate sanitary infrastructure represent high-risk settings for exposure to enteric pathogens, though the great majority of sanitation-related exposure and health outcome research has been focused on rural communities where sanitation coverage is lowest and open defecation is common. In the context of the Maputo Sanitation (MapSan) trial (2)(ClinicalTrials.gov Identifier: NCT02362932), we conducted a baseline, cross-sectional survey of compounds (defined as multi-household clusters with shared outdoor space) served by shared latrines. The aim of our study was to estimate prevalence of selected enteric pathogens in stool samples of enrolled children from this cohort, and to identify WASH and other risk factors for enteric infections.

METHODS

Study design & health outcomes

This cross-sectional study measures enteric infections and key socio-demographic and WASH-related risk factors among children in low-income neighborhoods of Maputo. We defined four outcomes, based on analysis of stool for 15 common enteric pathogens, for our risk factor assessment: (1) detection of any enteric infection, (2) detection of any

bacterial infections, (3) detection of any protozoan infections, and (4) detection of any viral infections. We also measured caregiver-reported diarrhea with 7-day recall as a secondary outcome (70). We defined diarrhea as ≥3 loose or liquid stools in a 24-hour period or any stool with blood (71).

Study setting

The study sites are located in densely populated, low-income, unplanned neighborhoods of Maputo, Mozambique. Poor sanitary conditions, inadequate infrastructure, environmental conditions including seasonal flooding, and increasingly high population density in these areas has led to a high burden of enteric disease and child mortality (30,72). In 2015, an estimated 53% of the urban population in Mozambique (~4.5 million people) lacked access to basic 'improved' sanitation facilities, as defined by the UNICEF/WHO Joint Monitoring Program (73). In Maputo, approximately 89% of households use onsite waste disposal (10% have access to sewerage; an estimated 1% practice open defecation), and only 26% of fecal waste is safely managed (74). An estimated 8% of urban sanitation in Mozambique is shared, often among the poorest households in informal neighborhoods (1). All households in the MapSan trial used shared sanitation facilities that were in poor condition at the time of enrollment.

Enrollment

Field teams enrolled children and collected baseline data concurrently between February 2015 and February 2016. We enrolled all children who met the following eligibility criteria: (1) the child's parent or guardian provided written informed consent, (2) the child was 1 - 48 months of age at the time of enrollment, and (3) the child resided in

compounds meeting certain inclusion criteria. Compounds were eligible for enrollment if they were located within a predefined geographic area, were in close proximity to a legal piped water supply, had a minimum number of households (2), and residents shared sanitation in poor condition and had stated demand for improved sanitation. The larger MapSan trial involved additional criteria to select compounds for intervention and details are presented in Chapter 3. Our enrollment period overlapped with the September 2015 rollout of the rotavirus A vaccination program in Mozambique. Children six weeks or younger at the time of rollout and children born after rollout began were eligible for immunization; some children enrolled in our study after September 2015 may have received the vaccination.

Data collection

Following enrollment, field teams collected data on socio-demographics and WASH-related risk factors using questionnaires and direct observation. Questionnaires are available in Appendix C (Table C1, Table C2). Enumerators administered three levels of surveys in each compound with an enrolled child: compound-level, household-level, and child-level. For compound-level surveys, the head of compound or the head of compound's spouse was the target respondent. For household- and child-level surveys, the child's mother was the target respondent, though another parent or guardian was eligible to complete the questionnaire. All questionnaires were communicated in either Portuguese or the local language, Changana, as requested by the respondent.

Surveys included socioeconomic and demographic questions such as child age and sex, household assets, caregiver's education level, and breastfeeding practices. We calculated

household wealth using an asset-based wealth index developed for Mozambique (75). At each level, surveys included direct observations and questions about risk factors of enteric infection, including characteristics of household and compound level water and sanitation, sanitary condition of living spaces, presence of animals within the compound grounds, environmental conditions including flooding patterns, and measures of population density and crowding. We created a composite 'latrine improvement score' ranging from 0-4 with one point awarded for the presence of each of the following latrine features: permanent superstructure, tile or masonry slab, drop-hole cover, and ventilation pipe. Similarly, we created a "compound sanitary score" ranging from 0-3 with higher scores indicating poorer sanitary conditions. One point was awarded for each of the following potential risk factors: (1) compound floods during rainy season, (2) leaking or standing wastewater observed by latrine, and (3) feces or soiled diapers observed around compound grounds. Compound-specific population density was defined as the number of people who live in a compound divided by the area of that compound. We measured the area of the compound using high resolution, orthorectified and geolocated satellite imagery. Enumerators equipped with GPS enabled tablets would work with compound residents to identify landmarks and define the shape of a compound on the satellite imagery. We calculated compound area from the shapes and divided the number of compound residents by the calculated compound area to obtain our measure of compound-specific population density. We used rainfall data from the National Oceanic and Atmospheric Administration's National Centers for Environmental Information (https://www.ncdc.noaa.gov/cdo-web/datatools/findstation) to calculate cumulative rainfall during the 30 days before data collection.

Sample collection and laboratory analysis

We provided stool collection supplies, including diapers, plastic potties (for older children no longer wearing diapers), and pre-labeled sterile sample bags to the caregiver of each enrolled child. Samples were collected, irrespective of reported symptoms, the following day. If a specimen was not immediately available, caregivers alerted the field team by phone when available. Following collection, samples were stored on cold packs, and transported to the medical parasitology laboratory at the Mozambican Ministry of Health (MISAU/INS) within six hours of collection for storage at -80°C. If a child produced a liquid stool, lab technicians stored a piece of the soaked diaper material ("diaper samples") at -80°C upon receipt. Stool samples were shipped on dry ice with temperature probes to the Georgia Institute of Technology where they were stored at -80°C until analysis.

We used the Luminex MagPix xTAG Gastrointestinal Pathogen Panel (GPP, Luminex Corp, Austin, TX) to analyze stool samples for the presence of 15 enteric pathogens: *Campylobacter*; *Clostridium difficile*, Toxin A/B; Enterotoxigenic *Escherichia coli* (ETEC) LT/ST; Shiga-like toxin producing *E. coli* (STEC) stx1/stx2; *E. coli* O157, a serotype of STEC; *Salmonella*; *Shigella*; *Vibrio cholerae*; *Yersinia enterocolitica*; adenovirus 40/41; norovirus GI/GII; rotavirus A; *Giardia*; *Cryptosporidium*; and *Entamoeba histolytica*. The GPP is a stool-based multiplex RT-PCR assay that has been extensively tested for direct detection of enteric infections in a range of countries (76–84). Per GPP protocol, we pretreated bulk stool samples with 1 mL of ASL stool lysis buffer (Qiagen, Hilden, Germany) and performed nucleic acid extraction for DNA and

RNA using the QIAcube HT platform and the QIAamp 96 Virus QIAcube HT Kit (Qiagen, Hilden, Germany). We eluted diaper samples in 2.5 mL of ASL stool lysis buffer. A sterile 10-mL syringe was used to facilitate elution via agitation by taking in and expelling the buffer 5 times. We used 1 mL of the final eluate in the pretreatment step and then proceeded with extraction as previously described. Extracts were stored at 4°C and analyzed by GPP within 24 hours of extraction.

Data analysis

Sample size for the present study is based on enrollment in the larger MapSan trial. Sample size calculations for the larger MapSan trial have been described previously (2). To minimize potential bias, we specified the statistical model and variables of interest before beginning the analyses. Details for individual variables used in these analyses - including definitions, coding schemes and proportions of missing values - are available in Appendix B (Table B1).

We calculated unadjusted and adjusted risk ratios (RRs) and 95% confidence intervals for outcome variables and potential risk factors using generalized estimating equations (GEEs) to fit Poisson regression models with robust standard errors (85). We used GEEs to account for clustering at the compound level. Outcome variables, including any infection and infection with bacterial, protozoan, or viral pathogens, were defined to identify differences in exposure risks from pathogen groups with different dominant routes of transmission (e.g. person to person versus environment to person). All multivariable models were adjusted for a set of five variables determined *a priori* as contextually important covariates. These variables included child age and sex,

breastfeeding practices, caregiver's education level, and an index of household wealth. We also calculated RRs and aRRs for enteric infections using child age (stratified by age group: 1-11, 12-23, and 24-48 months), sex, breastfeeding practices, and caregiver's education as the predictors of interest. We ran separate multivariable models for each combination of risk factor and outcome and assessed multicollinearity of multivariable models using the variance inflation factor. We assessed crude and adjusted associations between specific enteric pathogens and diarrheal symptoms as described for the main risk factor analysis.

Our primary analysis focused on complete observations. The proportion of incomplete observations per variable are denoted in supporting information (S1 Table). In parallel with the complete case analysis, we ran all univariable and multivariable models on completed data following multiple imputation (MI) of missing values (86–89). Details of the MI process are presented in Appendix B (Supporting Information B1). Briefly, we performed MI using chained equations (also known as fully conditional specification) to handle missing data (88,90). MI models were congenial with previously discussed analysis models and included a fixed effect to account for clustering at the compound level. Auxiliary variables were included in the MI model if they were *a priori* defined as related to either an outcome or predictor, if they were correlated with observed values of an outcome or predictor ($r \geq 0.2$), or if they were correlated with missingness of any outcome or predictor variable ($r \geq 0.2$) (89). All statistical analyses were performed with Stata version 14.1 (StataCorp, College Station, TX).

Ethics Statement

The head of the compound provided verbal assent for study activities before enrollment of any children within the compound. As children were ≤4 years old at the time of visitation, field enumerators obtained written informed consent from each child's parent or guardian before enrollment. The study protocol was approved by the Comité Nacional de Bioética para a Saúde (CNBS), Ministério da Saúde (333/CNBS/14), the Ethics Committee of the London School of Tropical Medicine and Hygiene (reference # 8345), and the Institutional Review Board of the Georgia Institute of Technology (protocol # H15160). The associated MapSan trial has been registered at ClinicalTrials.gov (NCT02362932).

RESULTS

Enrollment

Field workers enrolled 519 of the 601 compounds approached regarding participation in the MapSan study. Eighty-two (15.8%) compounds were ineligible for enrollment because they did not have a child <48 months old at the time of visitation. From those 519 compounds, workers enrolled 993 children in 815 households. Field teams administered child-level surveys for 980 of the 993 (99%) enrolled children and collected stool samples from 759 (76%) (Appendix B, Table B1).

Sociodemographic characteristics and prevalence of risk factors among study children

The average age of enrolled children was 23 months (Table 1). Approximately 27% (258/944) were <12 months old, while an equal percentage (28%, 266/945) were 12-23 months old, and the remainder (45%, 421/944) were 24-48 months old. Breastfeeding was very common among children 1-11 months old (87%, 224/258), though 31% (82/266) of children 12-24 months were also breastfed. A little over half of child caregivers had completed primary school (527/980). About 17% (163/975) of households met an *a priori* definition of crowding (>3 people per room of living space).

Table 2: Baseline measures of demographic, socioeconomic, environmental, and WASH-related exposure variables presented as # participants (%).

	Total n	# (%)
Latrine wall present	974	305 (31)
Drop-hole cover	974	557 (57)
Vent pipe	975	138 (14)
Pedestal or slab	971	361 (37)
Latrine improve index (range 0-4), unitless, mean (SD)	953	1.41 (1.24)
Households per latrine drop-hole	950	
<=2		171 (18)
3-5		576 (61)
>5		203 (21)
Child feces disposal in latrine	980	289 (29)
Standing water observed	974	71 (7.3)
Waste water observed	974	606 (62)
Feces observed	974	455 (47)
Compound has tendency to flood	974	601 (62)
Compound sanitary score index, unitless, mean (SD)	974	1.71 (1.06)
Drinking water tap on compound grounds	976	757 (78)
Any animal present	993	645 (65)
Dog present	993	76 (7.7)
Ducks or chickens present	993	131 (13)
Cat present	993	550 (55)
Household floor is covered	975	917 (94)
>3 Persons per room (household crowding)	975	163 (17)
Child Age (days), mean (SD)	967	662 (390)
Child sex, female	967	500 (52)
Any breastfed	980	316 (32)
Caregiver completed primary education	980	527 (54)
Wealth index (unitless), mean (SD)	976	43.7 (10.2)

Definitions of variables present in Appendix B, Table B1.

Almost all study children lived in a household that had access to a latrine in the compound (98%, 956/973) and most had access to latrines (61%, 576/950) shared by 3-5 households (median = 4). About half of children had latrines with drop-hole covers (57%, 557/974), 37% (361/971) had a masonry or ceramic slab or pedestal, while only 31%

(305/974) had a formal superstructure (made of bricks or cement blocks), and 14% (138/975) had a vent pipe. Sanitary conditions of compounds were poor: 62% (606/974) of study children lived in compounds with wastewater leaking from in or around a latrine and 47% (455/974) lived in compounds where feces or soiled diapers were visible around the grounds. Disposal of child feces into a latrine was common for children 24-48 months old (57%, 238/421). Feces of children between the ages of 1 – 23 months, most of whom wore diapers, was less frequently disposed of in a latrine (6.4%, 34/528). Most children lived in study compounds with animals (65%, 645/993), with cats (55%, 550/993) most commonly observed. All study households used piped water as their primary drinking water source and 78% (757/976) of children lived in households with access to a drinking water tap on the compound grounds.

Prevalence of enteric infections in study children

One or more pathogens were identified in stool samples from 655 (~86%) of the 759 children from whom a sample was collected; most (59%, 445/759) had coinfections (Table 2). Stool samples from 66 (8.7%) children yielded four or more enteric pathogens. The prevalence of coinfection (≥2 infections) increased with age from 33% (69/208) in the youngest age group to 73% (214/293) in the oldest. Most children (76%, 579/759) had a bacterial infection, about half (53%, 402/759) had a protozoan infection, and only 14% (107/759) of children had a viral infection. *Giardia*, *Shigella*, ETEC, *Salmonella*, and norovirus were the most frequently detected pathogens among all children, though prevalence varied with age. Prevalence of any infection, and of bacterial and protozoan infections by themselves, increased with age and were largely driven by the most

common bacterial and protozoan infections: *Shigella* and *Giardia*. Prevalence of *Shigella* infection increased from 9% (19/208) in children 1-11 months old to 65% (189/293) of children aged 24-48 months. *Giardia* showed a similar pattern with prevalence increasing from 14% (29/208) among 1-11 month-olds to 75% (219/293) prevalence in 24-48 month-olds. Prevalence of viral infections, largely driven by norovirus GI/GII, was highest among the youngest children (17%, 36/208) and lowest among the oldest children (11%, 33/293). Prevalence of rotavirus was low among all age groups (1-2%). Prevalence of enteric infections was similar among boys and girls with the exception of viral infections which tended to be more frequent in girls (17%, 64/370) than boys (11%, 41/370). Only 13% (126/980) of children were reported to have had diarrhea in the previous week. Reported diarrhea was higher among boys (16%, 74/464) than girls (10%, 50/498) and peaked in children aged 12-23 months (20%, 52/266). Norovirus was the only infection associated with higher risk of reported diarrhea (adjusted RR (aRR): 1.76, 95% CI: 1.03 – 3.02 adjusted for child age and sex, caregiver education, breastfeeding practices, and household wealth (Appendix B, Table B2), aRR 1.75, 95% CI: 1.00-3.1 when also adjusted for presence of all other measured pathogens).

Table 3. Prevalence and 95% confidence intervals of enteric infections in children <4 years of age measured at baseline.

	All, n=759	Female, n=370	Male, n=367	1-11 months, n=208	12-23 months, n=225	24-48 months, n=293
Any Infection (≥1 infections)	0.86 (0.84-0.89)	0.88 (0.84-0.91)	0.85 (0.81-0.89)	0.71 (0.64-0.77)	0.87 (0.82-0.91)	0.96 (0.93-0.98)
Any Viral Infection	0.14 (0.12-0.17)	0.17 (0.14-0.22)	0.11 (0.08-0.15)	0.17 (0.12-0.23)	0.15 (0.11-0.20)	0.11 (0.08-0.15)
Any Bacterial Infection	0.76 (0.73-0.79)	0.78 (0.74-0.82)	0.74 (0.69-0.78)	0.65 (0.58-0.72)	0.74 (0.68-0.80)	0.84 (0.79-0.88)
Any Protozoan Infection	0.53 (0.49-0.57)	0.52 (0.47-0.57)	0.54 (0.49-0.60)	0.18 (0.13-0.24)	0.53 (0.47-0.60)	0.76 (0.71-0.81)
Number of coinfections						
≥2 infections	0.59 (0.55-0.62)	0.62 (0.56-0.67)	0.55 (0.50-0.60)	0.33 (0.27-0.40)	0.60 (0.53-0.66)	0.73 (0.68-0.78)
≥3 infections	0.27 (0.24-0.31)	0.29 (0.24-0.34)	0.25 (0.21-0.30)	0.13 (0.09-0.19)	0.33 (0.27-0.39)	0.32 (0.27-0.38)
≥4 infections	0.09 (0.07-0.11)	0.10 (0.07-0.14)	0.08 (0.05-0.11)	0.04 (0.02-0.08)	0.14 (0.10-0.19)	0.08 (0.05-0.12)
Bacteria						
Shigella	0.44 (0.40-0.48)	0.44 (0.39-0.49)	0.43 (0.38-0.48)	0.09 (0.06-0.14)	0.44 (0.38-0.51)	0.65 (0.59-0.70)
ETEC LT/ST	0.30 (0.27-0.34)	0.32 (0.27-0.37)	0.28 (0.24-0.33)	0.23 (0.18-0.29)	0.37 (0.31-0.44)	0.30 (0.25-0.35)
Salmonella	0.21 (0.18-0.24)	0.22 (0.18-0.26)	0.19 (0.15-0.24)	0.29 (0.23-0.36)	0.20 (0.16-0.26)	0.16 (0.12-0.20)
Campylobacter	0.08 (0.06-0.10)	0.08 (0.06-0.12)	0.08 (0.05-0.11)	0.10 (0.06-0.15)	0.09 (0.06-0.13)	0.05 (0.03-0.09)
Clostridium difficile, Toxin A/B	0.05 (0.03-0.06)	0.05 (0.03-0.08)	0.05 (0.03-0.07)	0.11 (0.07-0.16)	0.04 (0.02-0.08)	0.01 (0.00-0.02)
Escherichia coli O157	0.04 (0.03-0.06)	0.05 (0.03-0.08)	0.03 (0.01-0.05)	0.03 (0.01-0.06)	0.04 (0.02-0.08)	0.05 (0.03-0.08)
STEC stx1/stx2	0.02 (0.01-0.03)	0.02 (0.01-0.05)	0.01 (0.00-0.03)	0.01 (0.00-0.04)	0.03 (0.01-0.06)	0.01 (0.00-0.03)
Yersinia enterocolitica	0.00 (0.00-0.01)	0.00 (0.00-0.01)	0.00 (0.00-0.01)	0.00 (0.00-0.02)	0.00 (0.00-0.02)	0.00 (0.00-0.01)

Table 3 (continued).

Vibrio cholerae	0.00 (0.00-0.00)	0.00 (0.00-0.01)	0.00 (0.00-0.01)	0.00 (0.00-0.02)	0.00 (0.00-0.02)	0.00 (0.00-0.01)
Protozoa						
Giardia	0.51 (0.48-0.55)	0.51 (0.46-0.56)	0.52 (0.47-0.58)	0.14 (0.10-0.19)	0.53 (0.46-0.60)	0.75 (0.69-0.80)
Cryptosporidium	0.03 (0.02-0.05)	0.03 (0.01-0.05)	0.04 (0.02-0.06)	0.05 (0.02-0.09)	0.04 (0.02-0.08)	0.02 (0.01-0.04)
Entamoeba histolytica	0.01 (0.00-0.01)	0.00 (0.00-0.01)	0.01 (0.00-0.02)	0.00 (0.00-0.03)	0.00 (0.00-0.02)	0.01 (0.00-0.03)
Virus						
Norovirus GI/GII	0.10 (0.08-0.13)	0.13 (0.09-0.17)	0.08 (0.06-0.12)	0.13 (0.09-0.18)	0.11 (0.07-0.16)	0.08 (0.05-0.12)
Adenovirus 40/41	0.03 (0.02-0.04)	0.05 (0.03-0.07)	0.01 (0.00-0.03)	0.03 (0.01-0.07)	0.03 (0.01-0.06)	0.03 (0.01-0.05)
Rotavirus A	0.01 (0.01-0.02)	0.01 (0.00-0.03)	0.01 (0.00-0.03)	0.01 (0.00-0.04)	0.02 (0.01-0.05)	0.01 (0.00-0.02)
Self-Reported Diarrhea	0.13 (0.11-0.15) n=980	0.10 (0.08-0.13) n=498	0.16 (0.13-0.20) n=464	0.14 (0.10-0.19) n=258	0.20 (0.15-0.25) n=266	0.09 (0.06-0.12) n=421

Risk of any enteric infection in unadjusted and adjusted models

Risk factors for enteric infection were assessed using generalized estimating equations in unadjusted models and models adjusted for age and sex of child, socioeconomic status, caregiver's education, and any breastfeeding. Among complete cases (Table 4), presence of a latrine superstructure was associated with 7% reduced risk of any enteric infection in the unadjusted model (risk ratio (RR): 0.93, 95% CI: 0.86-1.00), though the association was attenuated in adjusted models (RR: 0.95, 95% CI: 0.89-1.02). Presence of visible feces or used diapers in the compound was a risk factor in both unadjusted and adjusted models (aRR: 1.07, 95% CI: 1.01-1.14). Compound-specific population density was also associated with higher risk of ≥1 enteric infection; children living in the most densely populated quintile of compounds had a 10% higher risk (aRR: 1.10, 95% CI: 1.00-1.21) of any enteric infection compared with children in the least densely populated compounds. Among *a priori* covariates adjusted for in models, any breastfeeding was associated with a 13% reduced risk of any infection in adjusted models. Child age was positively associated with enteric infection; children in the oldest age group were 1.21 times more likely to have an enteric infection than children in the youngest age category. Risk factors for the any infection were also assessed by multiple imputation (Appendix B, Table B3) and results were consistent with complete case analysis (Table 4).

Table 4. Crude and adjusted risk ratios and 95% confidence intervals for associations of WASH-related risk factors and four measures of enteric infection: any enteric infection, any bacterial infection, any protozoan infection, and any viral infection. Multivariable models are adjusted for child age and sex, caregiver education, household wealth, and breastfeeding practices.

	Any enteric infection		Any bacterial infection		Any parasitic infection		Any viral infection		n
	RR	aRR	RR	aRR	RR	aRR	RR	aRR	UV; MV
Latrine superstructure	0.93 (0.86-1.00)*	0.95 (0.89-1.02)	0.95 (0.86-1.04)	0.96 (0.87-1.06)	0.80 (0.67-0.94)*	0.86 (0.74-1.01)	0.85 (0.56-1.29)	0.89 (0.58-1.35)	747; 704
Drop-hole cover present	0.95 (0.89-1.00)	0.96 (0.90-1.01)	0.90 (0.82-0.98)*	0.90 (0.83-0.99)*	0.93 (0.81-1.07)	0.96 (0.85-1.09)	1.00 (0.69-1.47)	0.92 (0.62-1.36)	740; 712
Vent pipe present	1.00 (0.92-1.08)	1.01 (0.93-1.10)	0.98 (0.86-1.12)	0.98 (0.85-1.12)	0.94 (0.74-1.18)	0.98 (0.80-1.21)	1.06 (0.64-1.78)	1.17 (0.72-1.89)	741;713
Pedestal or slab present	0.96 (0.90-1.03)	0.97 (0.91-1.03)	1.01 (0.92-1.10)	1.00 (0.91-1.09)	0.94 (0.79-1.10)	0.92 (0.80-1.07)	1.03 (0.70-1.50)	0.93 (0.63-1.37)	737; 709
Latrine improvement score	0.97 (0.95-1.00)*	0.98 (0.96-1.00)	0.97 (0.94-1.01)	0.97 (0.94-1.01)	0.94 (0.88-1.00)*	0.96 (0.90-1.01)	0.99 (0.86-1.14)	0.96 (0.83-1.12)	726; 698
HHs sharing latrine									728; 685
HH<=2	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	
3-5 HH	0.95 (0.89-1.03)	0.96 (0.90-1.03)	0.95 (0.86-1.05)	0.97 (0.88-1.07)	0.96 (0.80-1.16)	0.99 (0.83-1.18)	1.00 (0.61-1.62)	0.98 (0.59-1.62)	
> 5 HH	0.93 (0.85-1.02)	0.97 (0.89-1.05)	0.89 (0.78-1.02)	0.93 (0.81-1.06)	0.97 (0.77-1.21)	1.11 (0.90-1.37)	0.95 (0.51-1.77)	0.97 (0.51-1.84)	
Disposal of child feces in latrine	1.16 (1.10-1.22)*	1.01 (0.96-1.06)	1.17 (1.07-1.26)*	1.03 (0.93-1.13)	1.76 (1.55-1.99)*	1.08 (0.94-1.23)	0.78 (0.50-1.22)	0.93 (0.56-1.55)	746; 714
Standing water in compound	0.99 (0.90-1.10)	0.99 (0.89-1.10)	0.97 (0.81-1.16)	0.96 (0.80-1.16)	1.13 (0.94-1.36)	1.08 (0.86-1.34)	0.71 (0.35-1.42)	0.75 (0.38-1.48)	747; 704

Table 4 (continued).

Wastewater in compound	1.05 (0.99-1.12)	1.05 (0.99-1.12)	1.06 (0.97-1.16)	1.06 (0.97-1.16)	1.09 (0.93-1.27)	1.15 (0.99-1.32)	1.10 (0.75-1.63)	1.12 (0.75-1.68)	747; 704
Visible feces or used diapers	1.08 (1.01-1.14)*	1.07 (1.01-1.14)*	1.07 (0.98-1.16)	1.08 (0.99-1.17)	1.12 (0.97-1.29)	1.16 (1.01-1.32)*	0.85 (0.58-1.24)	0.95 (0.65-1.39)	747; 704
Compound floods when it rains	0.98 (0.92-1.04)	0.99 (0.93-1.05)	0.96 (0.88-1.05)	0.98 (0.90-1.07)	0.92 (0.80-1.07)	0.92 (0.81-1.06)	1.15 (0.78-1.69)	1.23 (0.82-1.84)	747; 704
Compound sanitary score	1.02 (0.99-1.05)	1.02 (1.00-1.06)	1.02 (0.98-1.06)	1.02 (0.98-1.07)	1.03 (0.96-1.10)	1.04 (0.98-1.11)	1.01 (0.86-1.19)	1.06 (0.89-1.25)	747; 704
Drinking water tap on compound grounds	0.97 (0.91-1.03)	0.97 (0.91-1.03)	0.97 (0.88-1.06)	0.97 (0.88-1.07)	0.88 (0.74-1.03)	0.85 (0.73-0.98)*	0.77 (0.51-1.17)	0.89 (0.58-1.36)	742; 714
Any animal in compound	1.02 (0.95-1.08)	1.02 (0.95-1.08)	1.03 (0.94-1.12)	1.04 (0.95-1.13)	0.98 (0.84-1.13)	0.95 (0.82-1.08)	1.41 (0.92-2.18)	1.46 (0.93-2.31)	759; 714
Dogs in compound	0.98 (0.89-1.08)	0.98 (0.89-1.08)	1.09 (0.98-1.22)	1.10 (0.98-1.22)	0.83 (0.60-1.15)	0.82 (0.61-1.10)	1.25 (0.65-2.43)	1.24 (0.68-2.25)	759; 714
Chickens or ducks in compound	1.02 (0.94-1.10)	0.99 (0.92-1.08)	1.00 (0.89-1.13)	1.00 (0.89-1.13)	1.05 (0.87-1.28)	0.97 (0.83-1.15)	0.93 (0.53-1.64)	0.94 (0.54-1.64)	759; 714
Cats in compound	1.03 (0.97-1.09)	1.03 (0.97-1.09)	1.03 (0.95-1.12)	1.04 (0.95-1.13)	1.00 (0.87-1.15)	0.95 (0.86-1.08)	1.33 (0.89-1.98)	1.35 (0.90-2.03)	759; 714
HH floor is covered	0.94 (0.84-1.04)	0.97 (0.88-1.08)	0.96 (0.82-1.13)	1.02 (0.86-1.21)	0.84 (0.64-1.10)	0.85 (0.70-1.03)	0.58 (0.34-1.00)*	0.59 (0.31-1.09)	741; 713
Household crowding, > 3 persons/room	1.00 (0.93-1.08)	0.98 (0.91-1.06)	1.04 (0.94-1.16)	1.02 (0.91-1.14)	0.88 (0.72-1.07)	0.82 (0.68-0.99)*	1.48 (0.98-2.26)	1.52 (0.95-2.43)	741; 713

Table 4 (continued).

Compound specific population density									740; 695
1 (least dense)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	
2	1.08 (0.97-1.120)	1.07 (0.96-1.18)	1.05 (0.82-1.21)	1.05 (0.91-1.21)	1.07 (0.85-1.34)	1.09 (0.89-1.34)	1.42 (0.78-2.60)	1.39 (0.73-2.64)	
3	1.06 (0.95-1.17)	1.04 (0.94-1.16)	1.13 (0.99-1.28)	1.13 (0.98-1.29)	1.00 (0.79-1.27)	1.01 (0.81-1.26)	0.04 (0.54-2.02)	1.11 (0.57-2.16)	
4	1.07 (0.96-1.19)	1.05 (0.94-1.17)	1.04 (0.90-1.20)	1.03 (0.88-1.20)	1.07 (0.85-1.35)	1.04 (0.83-1.29)	1.41 (0.73-2.71)	1.35 (0.67-2.73)	
5 (most dense)	1.11 (1.01-1.23)*	1.10 (1.00-1.21)*	1.06 (0.93-1.22)	1.06 (0.92-1.23)	1.01 (0.80-1.28)	1.13 (0.93-1.39)	1.56 (0.83-2.91)	1.44 (0.74-2.78)	
Cumulative rainfall last 30 days, terciles									759; 714
1 (least rain)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	
2	0.98 (0.92-1.05)	0.98 (0.92-1.05)	0.93 (0.84-1.02)	0.92 (0.83-1.02)	1.00 (0.84-1.19)	0.95 (0.81-1.10)	1.06 (0.68-1.66)	1.09 (0.69-1.72)	
3 (most rain)	0.95 (0.88-1.02)	0.94 (0.88-1.02)	0.95 (0.86-1.06)	0.94 (0.84-1.04)	1.04 (0.88-1.23)	0.99 (0.85-1.16)	1.22 (0.77-1.95)	1.35 (0.85-2.14)	
Child age									726; 698
1-11 months	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	
12-23 months	1.21 (1.10 - 1.34)*	1.12 (1.00 - 1.26)	1.12 (0.99 - 1.27)	1.02 (0.87 - 1.19)	2.89 (2.08 – 4.03)*	2.41 (1.64 - 3.57)*	0.83 (0.53 - 1.30)	0.62 (0.35 - 1.10)	

Table 4 (continued).

24-48 months	1.34 (1.22 - 1.47)*	1.21 (1.07 - 1.36)*	1.28 (1.14 - 1.44)*	1.14 (0.97 - 1.34)	4.20 (3.07 - 5.75)*	3.20 (2.14 - 4.80)*	0.63 (0.41 - 0.98)*	0.48 (0.25 - 0.93)*	
Child sex, female	1.04 (0.98 - 1.10)	1.04 (0.99 -1.10)	1.06 (0.98 - 1.16)	1.07 (0.99 - 1.16)	0.95 (0.83 - 1.09)	0.98 (0.86 - 1.11)	1.53 (1.09 - 2.14)*	1.65 (1.17 - 2.31)*	737; 714
Any breastfeeding	0.78 (0.72-0.85)*	0.87 (0.79-0.96)*	0.81 (0.73-0.89)*	0.93 (0.82-1.05)	0.34 (0.27-0.43)*	0.49 (0.36-0.66)*	1.11 (0.76-1.61)	0.81 (0.47-1.37)	742; 714
Caregiver completed primary school	0.95 (0.90-1.01)	0.97 (0.92-1.03)	1.00 (0.92-1.09)	1.03 (0.95-1.13)	0.83 (0.72-0.95)*	0.89 (0.79-1.01)	1.17 (0.83-1.65)	1.18 (0.82-1.70)	746; 714

* $p<0.05$

Risk of bacterial infection in unadjusted and adjusted models

Risk factors for any bacterial infection were assessed as previously described. Among complete cases (Table 4), presence of a drop-hole cover in the latrine was associated with reduced risk of any bacterial infection (aRR: 0.90, 95% CI: 0.83-0.99). Among *a priori* covariates, any breastfeeding was associated with 19% reduced risk of bacterial infection in the unadjusted model but was not associated with bacterial infection risk in the adjusted model. Despite increasing prevalence of any bacterial infection with age, we found no association between age and bacterial infection in adjusted models. Results from multiple imputation models were consistent with models limited to complete cases (Appendix B, Table B3).

Risk of protozoan infection in unadjusted and adjusted models

Among complete cases (Table 4), presence of a latrine superstructure was associated with 20% reduced risk of any protozoan infection in the unadjusted model but was only marginally associated with reduced risk in the adjusted model (aRR: 0.86, 95% CI: 0.74-1.01). In adjusted models, presence of visible feces or used diapers was associated with higher risk of protozoan infection (aRR: 1.16, 95% CI: 1.01-1.32). Household crowding, as well as presence of a drinking water tap on the compound grounds, were associated with reduced risk of protozoan infection in adjusted models only (aRR: 0.85, 95% CI: 0.73-0.98 and aRR: 0.82, 0.68-0.99).

Among *a priori* covariates included in all models, any breastfeeding was associated with reduced risk of protozoan infection in both unadjusted and adjusted models (aRR: 0.49, 95% CI: 0.36-0.66). Caregiver completion of primary school was associated with 17%

reduced risk of protozoan infection in the unadjusted model but was only marginally associated in the adjusted model (aRR: 0.89, 95% CI: 0.79-1.01). Age was a risk factor for protozoan infection; children in the 12-23 month and 24-48 month age groups had a 2.41 (1.64 – 3.57) and 3.20 (2.14 – 4.80) times higher risk of protozoan infection, respectively, than children aged 0-11 months (Table 3).

Among multiple imputation models, most results were in agreement with those in models limited to only complete cases (Appendix B, Table B3). The presence of visible feces or used diapers around the compound grounds was not associated with increased risk of protozoan infection in unadjusted or adjusted multiple imputation models.

Risk of viral infection in unadjusted and adjusted models

Viral infections were not associated with any of the risk factors assessed in adjusted complete case analysis (Table 4). Household crowding (presence of >3 persons per room) was only marginally associated with risk of any viral infection in adjusted models (aRR: 1.55, 95% CI: 0.95-2.43). Among *a priori* covariates, sex was a predictor of viral infection, with girls at higher risk of infection than boys (aRR: 1.65, 95% CI: 1.17-2.31). Children in the oldest age group (24-48 months) had 52% reduced risk of any viral infection compared with the youngest age group (1-11 months).

Results from multiple imputation models were consistent with results from models limited to only complete cases (Appendix B, Table B3). Among risk factors in multiple imputation models, household crowding was a risk factor in the unadjusted model (RR:

1.55, 95% CI: 1.04-2.32), but not in the adjusted model. Sex remained a risk factor for viral infection in both unadjusted and adjusted MI models.

DISCUSSION

We observed a high prevalence of enteric infection, including coinfections, among study children yet most children lacked diarrheal symptoms. The prevalence of enteric infection, but not reported diarrhea, increased with age though pathogen-specific age-related patterns varied. We found some independent WASH or environmental risk factors to be associated with enteric infection, though magnitudes of specific associations were often small. In this setting where burden of disease was high and sanitary conditions were poor, pathogen acquisition, symptomology, and the duration of carriage (colonization), may be driven by multiple interdependent risk and protective factors, including acquired immunity.

These results are consistent with findings from other studies of enteric infection in resource-constrained but predominantly rural settings in Africa and elsewhere. The Global Enteric Multicenter Study (GEMS) site in the rural district of Manhiça, Mozambique identified one or more enteric pathogens in 85% of stools from children with moderate-to-severe diarrhea (MSD) and 76% of stools from control children (without diarrhea in the 7 days preceding enrollment) (44). Similar trends were observed in the Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project (MAL-ED) study sites where 77% diarrheal and 65% of non-diarrheal stool samples were positive for ≥1 enteric pathogen (46). Studies using the GPP for enteric pathogen detection in similar settings in

Ghana and Côte d'Ivoire have also found high prevalence of enteric infection among both symptomatic and asymptomatic children (76,78).

Compared with enteric infection, the prevalence of caregiver-reported diarrhea was low. We observed a decrease in caregiver-reported diarrhea in children aged 24-48 months compared with the younger age strata, similar to the pattern observed for viral infections. Decreases in reported diarrhea follows a trend observed in historic data of hospital admissions for acute diarrheal episodes among young children in Mozambique (44). Though we could not formally calculate attributable fractions for etiologic agents of reported diarrhea with these data, we note that norovirus GI/GII was the only enteric pathogen associated with reported diarrhea. This is consistent with findings from the MAL-ED study sites where norovirus GII had one of the highest attributable fractions of diarrhea in children <2 years old (46). In contrast with reported diarrhea and viral infection, prevalence of bacterial and protozoan infections tended to increase with age, though patterns varied by pathogen. The high prevalence observed here, especially in older children, could be due to the poor clearance and accumulation of persistent enteric infections over time (91) or could be a result of a high rate of reinfection due to frequent pathogen exposure (92). As children age and become increasingly mobile they interact with their environment more, potentially leading to high exposures to fecal contamination and increased enteric infection (93).

While the overall prevalence of enteric pathogens was similarly high among our study and sites in GEMS and MAL-ED, there were differences in the frequency of detection of specific enteric pathogens. *Giardia* (51%), *Shigella* (44%), ETEC (30%), *Salmonella*

(21%) and norovirus GI/GII (10%) were the most frequently detected pathogens in this cohort of children. *Giardia*, rotavirus, *Cryptosporidium*, *E. histolytica*, and enteroaggregative *E. coli* (EAEC) were the most common pathogens detected among cases and controls at the GEMS-Manhiça site, just 80 kilometers north of our study sites (44). Across all MAL-ED sites, the most frequently detected pathogens in diarrheal and non-diarrheal stools were *Campylobacter*, *Giardia*, EAEC, and norovirus GII (46). Notably, even though our data collection occurred largely before the rollout of the rotavirus vaccine in Mozambique in September 2015, we detected almost no rotavirus in our study population. This is in stark contrast to findings from the GEMS-Manhiça site where rotavirus was deemed one of the principal causative agents of MSD and was detected in up to 18% of controls (44). To further interrogate this difference, we tested the 8 rotavirus GPP-positive specimens and 84 randomly selected rotavirus GPP-negative specimens for the presence of rotavirus using the Premier Rotaclone (Meridian Bioscience, Cincinnati, OH, USA) in-vitro diagnostic fecal antigen enzyme-linked immunosorbent assay (ELISA) (94). Using the ELISA results as the reference, we calculated the GPP to have 100% sensitivity and 100% specificity for detection of rotavirus A antigen in our fecal specimens. The variations in detection frequencies of enteric pathogens across these studies could be due to differences in detection methods or may suggest that pathogen profiles vary across even limited geographical distances. Molecular reanalysis of the GEMS specimens yielded higher detection frequencies of many bacterial pathogens than the original culture-based methods (95). However, the GEMS reanalysis did not substantially change detection of *Cryptosporidium* or rotavirus, highlighting potential geographic differences.

Results from this risk factor analysis are consistent with previous studies identifying the build quality or physical characteristics of latrines as factors for increased risk of infection exposure (96); we found presence of a superstructure or a drop-hole cover to be associated with decreased infection risk. We did not identify any association between enteric infection prevalence and the presence of a cleanable slab, however, consistent with previous work from Tanzania (97). Associations between the physical characteristics of a latrine and enteric infections were observed only for risk of bacterial and protozoan infections. Household crowding was also associated with a reduced risk of protozoan infection, further evidence that transmission of enteric bacterial and protozoan pathogens is likely to be largely environmentally mediated (9,12,13). We did not identify any WASH or environmental variables associated with risk of viral enteric infection. This is consistent with our prior assumption that person-to-person transmission is likely the predominant pathway for viral infection in this setting (2) as has been observed elsewhere under similar conditions (13).

Consistent with previous work, any breastfeeding appeared protective for enteric infection risk in our analysis (98–103). Adjusted estimates of association show that this observation is primarily driven by protection from *Giardia* infection (RR = 0.50, 95% CI 0.37 – 0.67); a similar correlation was also observed in the MAL-ED study (101). Any breastfeeding limits enteric pathogen transmission by eliminating exposure via direct consumption of contaminated food or water.

Maputo, like many cities of sub-Sahara Africa, is rapidly urbanizing (104). Urbanization may result in higher risk of direct (person-to-person) or indirect (environmentally-

mediated) transmission of enteric infection, especially in low-income, unplanned neighborhoods where WASH infrastructure is lacking (105,106). Recent studies of population density and enteric infection risk have found mixed results, though most were based in rural areas or less dense urban settings (107–109). In our study, we observed an association between higher compound-level population density and higher risk of enteric infections.

There are important limitations to this study that qualify our results. First, our *a priori* selection of specific pathogen targets and our methods for stool sample analysis present key constraints to interpretation. The GPP tests for 15 of the most common enteric pathogens including bacteria, viruses, and protozoa, but this is a sub-set of all enteric infections and therefore an incomplete accounting of current infections. For example, the GPP does not detect EAEC, a pathogen commonly detected in young children in both MAL-ED and the GEMS-Manhiça site (44,46) and associated with malnutrition (102). Metagenomics or other primer-independent approaches may have yielded information on additional targets of public health significance. Although detection of pathogens in stool samples was observed to be closely associated with age – suggesting persistent infections or frequent reinfection – we cannot make conclusions about either duration of infections or shedding or about the potential for rapid clearance and reinfection based on a single stool specimen. Detection of an enteric pathogen in stool can represent symptomatic or asymptomatic infection, pathogen carriage due to colonization of the gut, or simply passage due to recent exposure. Further, certain pathogens may be shed for weeks after clinical symptoms of infection have abated, and the onset or absence of symptoms

following infection can depend on factors related to the environment, host, or pathogen strain of interest (91). The GPP was designed to aid in diagnosis of enteric infections and the relatively high limits of detection ($2.2x10^2$ – $3.75x10^6$ CFU or copies/mL stool) largely exceed the known infectious doses for target pathogens. This suggests that enteric pathogen detections via the GPP may primarily represent active infection (symptomatic or asymptomatic) or long or short term colonization of the intestinal tract. Although detection of enteric pathogens in feces is an unambiguous indication of past exposure and a clear indication that fecal waste from such individuals represents downstream exposure risks, absence of a particular pathogen in stool by the methods we used does not indicate absence of previous exposure to that pathogen. Because the detection limit of the assay we used is relatively high, a negative assay may not necessarily mean that the pathogen is absent in stool. Cross-sectional, end-point RT-PCR analysis of stool samples alone cannot reveal information on time since exposure, etiology of symptomatic infections, intensity of infections, health implications of infections, or infectivity of pathogens shed in stool. Enteric infections are on the causal pathway between exposures and all downstream health impacts of WASH, including diarrheal disease and environmental enteric dysfunction, but they should be considered an intermediate outcome of uncertain clinical significance.

Second, the study population and the study setting, though diverse across some variables, was characterized by a limited range of WASH conditions. All participating households had access to shared sanitation without safe excreta management – a key criterion used in determining eligibility for the MapSan trial – and so exposures were likely to be high

across our study sites. This lack of heterogeneity of WASH conditions may have limited our ability to observe variation in risk attributable to specific exposures.

Third, certain inclusion criteria may limit the generalizability of our findings. Because our study only included children living in households sharing sanitation in densely populated urban neighborhoods, our results may not represent risks for children in rural areas or in households using private sanitation.

Fourth, our analysis is constrained by missing data for variables, including the outcome. A secondary analysis used multiple imputation to handle missing values, and these methods are accompanied by different assumptions and limitations. We note, however, that results from the complete case models and estimates from multiple imputation were largely consistent. Finally, our modeling strategy did not include adjustment for multiple comparisons. While it is possible that some of our findings are spurious and due to type I error (110,111), all variables in this analysis have strong foundations in the literature or plausibility as risk factors for enteric infection.

Overall, we found high prevalence of enteric infection and comparatively low prevalence of reported diarrhea among children <4 years old living in informal neighborhoods of Maputo, Mozambique. Most infections were observed in reportedly asymptomatic children. Prevalence of bacterial and protozoan infection increased with child age and is likely due to variations in exposure profiles as children become more mobile. Certain sanitation facility characteristics were associated with decreased risks of enteric infection, though the magnitude of these associations was small. The importance of effective sanitation increases where prevalence of enteric infections is high: fecal wastes in such

settings present elevated exposure risks, potentially driving burdens of infection and disease higher. Strategies to interrupt this cycle of infection and exposure risk should limit the possibility of exposure to excreta, including through multiple pathways of transmission.

CHAPTER 3

EFFECTS OF AN ON-SITE SHARED SANITATION INTERVENTION ON CHILDHOOD ENTERIC INFECTION AND DIARRHEA IN URBAN MOZAMBIQUE

ABSTRACT

Background

Exposure to fecal contamination due to inadequate sanitation can result in infection with enteric pathogens and short- and long-term health and well-being effects. Exposure to enteric pathogens may be frequent in low-income urban settings with high population density and low coverage of safe sanitation. We aimed to evaluate whether an on-site, privately shared sanitation intervention could reduce the burden of enteric infection and diarrheal disease in young children living in Maputo, Mozambique.

Methods & findings

The Maputo Sanitation trial is an independent controlled before-and-after study of the impact on an on-site sanitation intervention on child health in urban Mozambique. We enrolled and collected baseline (pre-intervention) data from children <4 years old in intervention and control sites between February 2015 and February 2016 and returned to collect 12-month and 24-month follow-up data between March 2016-April 2017 and April 2017-May 2018, respectively. Water and Sanitation for the Urban Poor (WSUP)

selected sites for intervention and designed and constructed the intervention latrines, pour-flush toilets to septic tanks with soakaway pits. The MapSan team, independent from WSUP, used a modified version of WSUP's site selection criteria to identify and enroll control sites. Enrollment was progressive and all eligible children were enrolled at each visit regardless of availability at baseline. In our main analysis of all enrolled children, the intervention had no impact on our pre-defined primary outcome, infection with one or more bacterial or protozoan infection as detected in stool by the Luminex Gastrointestinal Pathogen Panel (GPP) at either the 12- or 24-month time-point or when time-points were combined into a single follow-up phase. The intervention also had no measurable effect on the risk of infection with any individual pathogen target detected by the GPP. In a sub-group analysis of children born into intervention sites (post-intervention) by the 24-month follow-up time-point, the intervention reduced the risk of *Shigella* infection by 47% (aRR 0.53, 95% CI 0.29-0.95) but did not affect the risk of infection with any other individual pathogen. A second longitudinal sub-group analysis of children available at baseline and at least one follow-up phase also demonstrated no effect of the intervention on any enteric infection outcome. The intervention had no effect on reported diarrhea in the main analysis of all children or in either of the two sub-group analyses.

Conclusions

The intervention had a limited effect on the risk of on enteric infection and caregiver-reported diarrhea. In this and similar settings where infection burden is high and fecal contamination of the environment is pervasive, exposure is likely to be complex and

driven by multiple transmission pathways. To achieve rapid health gains, interventions may need to be transformative and address most, if not all, pathways of transmission in a given setting.

INTRODUCTION

Currently, 2.2 billion people in urban areas lack access to safely managed sanitation systems and this problem will only worsen as migration to urban areas accelerates over coming decades (3). Rapid urbanization will likely result in the expansion of informal urban settlements, or slums, in many areas, stressing existing inadequate sanitation systems and increasing the number of people without access to basic or safely managed sanitation (3,4). Exposure to fecal contamination due to lack of access to adequate sanitation has been associated with enteric infection, diarrheal disease, environmental enteric dysfunction (EED), linear growth deficits, impaired cognitive development, and other long term health and well-being effects (6,15–21,101,112). Children living in densely populated slum areas where fecal contamination is pervasive, infection burdens are high, and sanitation infrastructure is limited, may be at increased risk enteric infection and other down-stream sanitation-related health effects (24,25,105,113,114).

While development or expansion of centralized sanitation systems in urban areas has been demonstrated to have important health benefits and is an aspirational solution

(26,31,115–117), such systems may not be a practical fix in the short-term due to cost, logistical constraints, and the urgency of the need for sanitation solutions in many places (26,29). On-site systems which use soak-way pits for liquid effluent discharge may fill the growing need for safe sanitation in rapidly expanding urban areas in LMICs but, to date, there has been little evidence of the health impact such systems may provide in these settings. Several large-scale, rigorous evaluations of on-site sanitation interventions and combined water, sanitation, and hygiene interventions have been completed recently, however, all were performed in rural areas and their findings may have limited generalizability to urban areas (32–37). Further, results from these trials have demonstrated mixed effects on health. A recent meta-analysis estimated non-sewerage interventions reduced risk of self-reported diarrhea by 16%, however, it did not include results from the most recent studies (32,33,37) and did not estimate effects on objective health outcomes (31). Self-reported diarrheal disease is subjective and, when coupled with unblinded interventions, presents significant risk of bias as an outcome measure in health impact trials (118–120).

The Maputo Sanitation (MapSan) is the largest controlled trial of decentralized urban sanitation performed to date, the first to evaluate shared sanitation, and the first to use enteric infection, an objective measure of exposure to sanitation-related pathogens, as the primary study outcome (2). Our study is located in densely populated, low-income neighborhoods of Maputo, Mozambique where sanitary conditions are poor and disease burden is high (Chapter 2). As of 2017, an estimated 48% of urban residents in Mozambique lacked access to even basic sanitation infrastructure (1). In Maputo, the

capital city, only 10% of residents have access to sewerage, 89% rely on on-site disposal, and 1% practice open defecation (121). An estimated 9% of urban residents share sanitation with multiple households (1), often in the poorest neighborhoods where space and resources are limited. We aimed to investigate whether an on-site, privately shared sanitation intervention could reduce enteric infection and reported diarrhea in young children living in low-income, densely populated neighborhoods in urban Mozambique.

METHODS

Study design and intervention

The MapSan Trial is a prospective, non-randomized controlled before-and-after (CBA) study to measure the impact of an on-site, privately shared sanitation intervention on several important metrics of child health. We conducted the study in 17 densely populated, low-income, and unplanned neighborhoods in Maputo, the largest city and capital of Mozambique. The trial consisted of three phases of data collection: an enrollment and baseline phase, a 12-month follow-up phase, and 24-month follow-up phase. Each phase took approximately one year to complete. We enrolled and visited intervention and control sites concurrently to limit any differential influence of seasonality on data collection and outcome measurement (Figure 3). We revisited intervention sites approximately 12-months (median 397 days) and 24-months (median 792 days) after the intervention latrines were opened for us and revisited control sites at a

similar rate (Figure 3). Each site consisted of a single compound: a group of related or unrelated households with shared outdoor living space, sanitation facilities, and sometimes food preparation and cooking areas. Compound sanitation facilities are shared among member households but they are not considered public latrines.

The sanitation interventions, pour-flush latrines to septic tanks, were designed and constructed by the non-governmental organization Water and Sanitation for the Urban Poor (WSUP) between 2015 and 2017. WSUP built a total of 450 new latrines in 11 neighborhoods of the Nlhamankulu district of Maputo, Mozambique. Two types of latrines were constructed and delivered at the compound level: communal sanitation blocks (CSBs) and shared latrines (SLs). The primary difference between CSBs and SLs is size. CSBs include multiple stalls with toilets and serve compounds of 21 or more people with one stall allocated per 20 beneficiaries. CSBs also include rainwater harvesting systems, elevated water storage tanks, a laundry facility, a shared water connection, and a well-drained area for bathing (Appendix A, Figure A2). Shared latrines are single-stall infrastructure serving fewer than 21 people (Appendix A, figure A3). All interventions, regardless of size, are pour-flush latrines to septic tanks with soakaway pits. The septic tanks for both CSBs and SLs were designed to require emptying after two years of use (Appendix A, Figure A1).

Participants

Children between 29 days and 48 months old were eligible for baseline enrollment in the MapSan study if we received written informed consent by a parent or guardian and if they lived in a selected intervention or control compound. As this study was an

independent impact evaluation of a pre-planned intervention, our study team was not involved with intervention site selection. WSUP, the implementation organization, worked with neighborhood-level government representatives to select sites for intervention based on factors related to demographics, engineering constraints, and WASH conditions. Specifically, sites were evaluated using the following criteria: (1) compound residents must share sanitation in poor condition as determined by a WSUP engineer, (2) compounds must be located in the pre-defined implementation neighborhoods, (3) a minimum number of beneficiaries (12 – 21 depending on latrine type) must reside in the compound, (4) compound residents must have stated demand for improved sanitation and must be willing to contribute to construction costs, (5) sites must have space for construction of a new facility, (6) sites must be accessible for both transportation of construction materials and tank-emptying activities, (7) compounds must have nearby access to a legal piped water supply, and (8) the groundwater level must be deep enough to support construction of the septic tank. Handover of the original tranche of 300 compounds began in February 2015 and continued through February 2016. A second tranche of compounds (150) began in November 2016 and continued through June 2017. Our study team selected control sites using WSUP criteria 1, 3, 4, and 7. We modified criterion 3 to select control compounds to have similar numbers of residents as intervention compounds so that the population distribution of intervention and control compounds would be comparable. Criterion 4 was presented as a hypothetical during control selection. Prospective intervention sites which did not meet engineering and construction constraints (criteria 5, 6, and 8) were eligible for recruitment as controls. Control sites outside of WSUP's pre-defined implementation area were also eligible for

recruitment so long as they met criteria 1, 3, 4, and 7. Additionally, control compounds were required to have at least one child less than four years old in residence.

Concurrent with latrine construction and handover, our field teams completed baseline enrollment and data collection between February 2015 and February 2016. We aimed to visit intervention compounds two weeks prior to the opening and handover of the new latrines. Interventions and control compounds were enrolled at approximately the same rate during all three study phases to avoid potential biases due to seasonality. Follow-up visits at years one and two were scheduled to be 12 months (±2 weeks) and 24 months (±2 weeks) from the date intervention compound members began using their latrines. Within two weeks of each 12-month or 24-month follow-up visit of an intervention compound, a control compound was revisited, thus maintaining similarity in the rates of visits between study arms across phases. Enrollment was progressive meaning all eligible, consented children were enrolled in the study during each visit (baseline, 12-month, and 24-month). Children who moved into the compound after baseline measurement and fewer than six months before the 12-month or 24-month visit were not eligible for enrollment during that phase given their limited exposure to their new compound. Children born into a study compound after baseline measurement were eligible for enrollment regardless of the length of their exposure to the compound so long as they met the age criterion (>29 days). During enrollments at 12- and 24-month visits, the upper age limit was increased to 60 months as our target age demographic was children less than five years.

The nature of the intervention infrastructure made it impossible to blind participants and field enumerators to intervention status. However, the MapSan study team and the WSUP implementation team were independent and included different individuals.

Procedures

At each phase – baseline, 12-month, and 24-month - field teams completed consent procedures, administered surveys, and collected biological specimens from enrolled children. We obtained verbal assent from the head of the compound or his or her spouse prior to starting study related activities at each site. The parents or guardians of each eligible child provided written, informed consent prior to child enrollment in the study. Field enumerators sought verbal assent for continuation in the study at the first visit of each subsequent phase. Enumerators planned to visit households with enrolled children twice during each phase, though more visits were sometimes necessary to complete specimen collection. On the first visit of each phase, enumerators completed consent procedures and administered child-, household-, and compound-level surveys (Appendix C, Tables C1-C5) at households with eligible children and delivered stool specimen collection supplies. The next day, field enumerators returned to collect specimens and complete any remaining surveys. The child's mother was the target respondent for child and household surveys, though the father or another guardian was also eligible. For compound-level surveys, the head of compound or his or her spouse was the preferred respondent. All study-related communication was in Portuguese or the local language, Changana, depending on the preference of the respondent.

Surveys included questionnaires and direct observation and were tailored to collect data on key socio-demographic factors, environmental conditions in the household and compound, and WASH practices and behaviors. We used an asset-based wealth index developed for Mozambique to calculate relative household wealth (75). Other survey variables have been described previously (Chapter 2, Appendix B, Table B2). Enumerators used the mwater smartphone application for collection of survey data (www.mwater.co).

Field enumerators attempted to collect stool specimens from each enrolled child at each phase independent of reported symptomology. Enumerators provided each caregiver with stool collection supplies, including diapers, a plastic potty if the child was no longer wearing diapers, and a pre-labeled sterile sample bag. Enumerators returned the next day to collect the specimens. If a specimen was unavailable during the scheduled pickup, caregivers called the field team, using phone credit provided by the study, as soon as one was available or if fresh collection supplies were needed. If field enumerators were unable to collect a bulk stool sample after multiple attempts, a registered nurse used an anatomically designed rectal swab (Copan Diagnostics Inc, Murrieta, CA, USA) to collect fecal material. Parents or guardians were required to complete a separate written consent procedure prior to collection of rectal swabs. Bulk stool specimens and rectal swabs were stored in coolers with cold packs and delivered to the medical parasitology laboratory at the Mozambican Ministry of Health (MISAU/INS) within six hours of collection. Upon receipt, laboratory technicians aliquoted bulk stools into several sterile tubes and storage them, and any rectal swabs, at -80°C. If a child produced a liquid stool,

lab technicians stored a piece of the soaked diaper material ("diaper samples") at -80°C. Stool samples were shipped frozen on dry ice with temperature probes to the Georgia Institute of Technology in Atlanta, Georgia, USA where they were stored at -80°C until analysis.

A member of the Mozambican National Deworming Campaign (NDC) worked in coordination with our study team to provide single dose albendazole (400 mg, 200 mg for children aged 6 – 12 months) to all eligible members of intervention and control study compounds following completion of each study phase. Eligibility was defined by the NDC guidelines and included compound members older than six months who were not pregnant at the time of distribution.

We analyzed stool specimens (bulk, diaper, and rectal swabs) for the presence of 15 enteric pathogens: *Campylobacter*; *Clostridium difficile*, Toxin A/B; Enterotoxigenic *Escherichia coli* (ETEC) LT/ST; Shiga-like toxin producing *E. coli* (STEC) stx1/stx2; *E. coli* O157, a serotype of STEC; *Salmonella*; *Shigella*; *Vibrio cholerae*; *Yersinia enterocolitica*; adenovirus 40/41; norovirus GI/GII; rotavirus A; *Giardia*; *Cryptosporidium*; and *Entamoeba histolytica* using the Luminex MagPix xTAG Gastrointestinal Pathogen Panel (Luminex Corp, Austin, TX). The Gastrointestinal Pathogen Panel (GPP) is a multiplex end-point reverse transcriptase polymerase chain reaction (RT-PCR) assay developed to aid diagnosis of enteric pathogen infection in clinical settings (122).The GPP has been rigorously and extensively tested for direct detection of enteric pathogens in stool in a range of settings and countries including some similar to the present study (76–84,123). The analysis of bulk stool, diaper specimens,

and rectal swabs have been previously described (Chapter 2). Briefly, we adhered to pretreatment, lysis, and analysis procedures described in the GPP protocol with additional elution steps included in the pretreatment procedure for diaper and rectal swab samples. We added MS2, a non-pathogenic RNA virus, to each sample prior to nucleic acid extraction as an extraction and RT-PCR inhibition control. We included at least one sample process control (containing only lysis buffer and MS2) and negative extraction control (containing only lysis buffer) with each set of extractions. During the PCR step, we included at least one no-template control, containing molecular grade water and all PCR reagents, with each run. To assess elution and extraction of nucleic acid from diaper and swab samples, we measured the concentration of double-stranded DNA (dsDNA) present in extracts using the Qubit® High Sensitivity dsDNA kit (Invitrogen™, Carlsbad, CA, USA) and Qubit® 4 Fluorimeter (Invitrogen™, Carlsbad, CA, USA). Following extraction we stored all extracts at 4°C and analyzed them by GPP within 24 hours. For long-term storage, we archived samples at -80°C. We extracted and analyzed approximately 10% of samples in duplicate. If duplicate analyses yielded different results, we combined the results from all analyses. If we could not detect a MS2 signal in a given sample, we either re-extracted or diluted the extract 1:10 in molecular grade water and re-assayed by GPP.

Outcomes

We pre-specified the primary outcome of the study as infection with one or more of the 12 bacterial or protozoan enteric pathogens detected by the GPP and a secondary outcome as caregiver reported diarrhea with a 7-day recall (64). We excluded viral

enteric pathogens from the primary outcome definition because we hypothesized that their dominant transmission pathway in this setting may be person-to-person, a pathway unlikely to be interrupted by the intervention. Other pre-specified outcomes - the results of which will be described elsewhere - include soil-transmitted helminth infection, concentrations of biomarkers of environmental enteric dysfunction (Chapter 4), and measures of growth and nutrition (height-for-age, weight-for-age, and height-for-weight z-scores and associated metrics of stunting, underweight, and wasting). In addition to the pre-specified primary outcome, we will also evaluate the effect of the intervention on specific pathogen types (bacterial, protozoan, viral) and on individual pathogen targets.

We defined caregiver reported diarrhea as the passage of the three or more loose or liquid stools in a 24-hour period or any stool with blood (70,71). Field enumerators assessed caregiver reported diarrhea in the preceding seven days as part of the child-level surveys. We measured reported diarrhea in all available, enrolled children at each phase.

Statistical analysis

Our initial sample size calculations were based on the planned construction of 190 intervention latrines. We estimated a necessary enrollment of 380 children per study arm assuming 80% power, a primary outcome risk ratio of 0.84 which assumed high prevalence of endemic infection in controls (70%), a small clustering effect (intraclass correlation coefficient (ICC) = 0.1) due to the low number of participants per cluster, and approximately 10% loss to follow-up (2,124). Even though the number of planned intervention latrines increased just prior to the start of the study, ultimately, due to much higher than anticipated loss to follow-up (~38% between baseline and 12-month), sample

size for this study was constrained by the number of intervention latrines constructed and the number of eligible children in those compounds.

The primary analyses included all enrolled children living in control compounds or in intervention compounds which received an intervention latrine during the baseline phase of the study (February 2015 – February 2016). Children living in compounds which received an intervention latrine after February 2016 are excluded from the current analyses but will be included in a future sub-group analysis. The primary analyses examined the effect of the intervention at the 12-month and 24-month phases separately. We also performed an exploratory effect analysis which combines all data collected after baseline into a single follow-up phase. This combined follow-up analysis assumes that the effect of the intervention was uniform over the study period.

Additional sub-group analyses examined here include a comparison of (1) children with baseline data and data from at least one phase of follow-up and (2) children who were born into a study site post-intervention (and aged <1 year at 12-month visit or less than 2 years at 24-month visit) or were < 1- 2 years at baseline (to obtain comparable age distributions for comparison).

We used chi-squared tests and two sample *t* tests to test for differences in baseline demographic, socio-economic, and environmental and WASH characteristics between the two study arms. We used a difference in difference (DID) approach to assess the impact of the intervention on infection and reported diarrhea outcomes at the 12- and 24-month phases. In regression analyses, the DID estimator is created as an interaction term of dummy variables representing the study arm and study phase. For binary outcome

measures, such as the primary outcome measure (defined as ≥1 bacterial or protozoan infection as detected by the GPP) or reported diarrhea, we used generalized estimating equations (GEE) to fit modified Poisson regression models and account for compound level clustering effects. For the count of the number of infections detected by GPP, we used GEE to fit a negative-binomial regression model since the distribution of count data was zero-inflated (125). We clustered on compound because it was the highest level of nested data (which included household level, child level, and repeated measures data) and the level of the intervention allocation (126). The primary outcome assessment is based on multivariable models which adjust for covariates determined *a priori* as potentially predictive of the primary outcome, including child age, sex, and breastfeeding status, caregiver's education, and household wealth. Additional covariates we considered for inclusion in multivariable models included measures of population and crowding (e.g. number of compound members, number of households, number of household members, compound-level population density), compound level amenities (e.g. functioning electricity), environmental conditions (e.g. propensity of compound to flood, cumulative rainfall during month prior to measurement, standing water, presence of animals), and baseline WASH conditions (presence of a pedestal or slab, drophole cover, vent pipe, or sturdy latrine walls, number of water taps and latrines in the compound). We excluded from consideration any variable with limited variation (<5%) in the study population at baseline. To select covariates for the multivariable model, we first assessed whether they were associated with the treatment assignment (study arm) and the primary outcome (or diarrhea) at baseline (>10% relative difference in prevalence or value). Covariates associated with the exposure (study arm designation) and outcome at baseline were

entered into univariable DID models to evaluate whether their inclusion resulted in a meaningful change in effect in the outcome (±10% change in DID estimator). No additional covariates met these criteria and our final multivariable models adjust for only the *a priori* covariates listed previously. We also present the results from the unadjusted, univariable models. The results of effect estimation are evaluated with traditional statistical hypothesis testing using a significance level of α=0.05. We did not adjust for multiple comparisons because the main analyses of the primary outcome and reported diarrhea were pre-specified and all others, including analysis of pathogen groups, individual pathogens, and all sub-group analyses were exploratory (127). We analyzed data from participants according to their exposure status (as treated analysis). Statistical methods for sub-group analyses were similar to those for the primary analysis. We performed all statistical analyses with Stata version 14.1 (StataCorp, College Station, TX).

Ethics

The study protocol was approved by the Comité Nacional de Bioética para a Saúde (CNBS), Ministério da Saúde (333/CNBS/14), the Ethics Committee of the London School of Tropical Medicine and Hygiene (reference # 8345), and the Institutional Review Board of the Georgia Institute of Technology (protocol # H15160). The trial has been registered at ClinicalTrials.gov (NCT02362932)

RESULTS

Enrollment

We enrolled 993 children in 497 compounds during the baseline phase (February 2015 – February 2016) (Figure 2). Children in intervention and control compounds were enrolled at approximately the same rate during baseline (Figure 3). Of these 993 children, 456 (45.9%) resided in 207 intervention compounds and 537 (54.1%) resided in 290 control compounds (Figure 2). We collected stool specimens from 362/456 (79%) children enrolled in intervention sites and 395/537 (74%) children enrolled in control sites at baseline. We also collected data on caregiver-reported diarrhea from 99% of children in intervention (450/456) and control (530/537) sites.

The 12-month follow-up phase lasted from March 2016 – April 2017, during which time we visited 458 children in 196 intervention compounds and 482 children in 242 control compounds. We collected stool material from 403/458 (88%) intervention children and 400/482 (83%) control children and data on caregiver-reported diarrhea from 432/458 (94%) intervention children and 435/482 (90%) control children. Sixty-six percent (303/456) of children enrolled into intervention compounds and 59% (317/537) of children enrolled into control compounds at baseline were available for follow-up at the 12-month visit. An additional 155 children were enrolled into the intervention arm and 165 into the control arm during the 12-month phase.

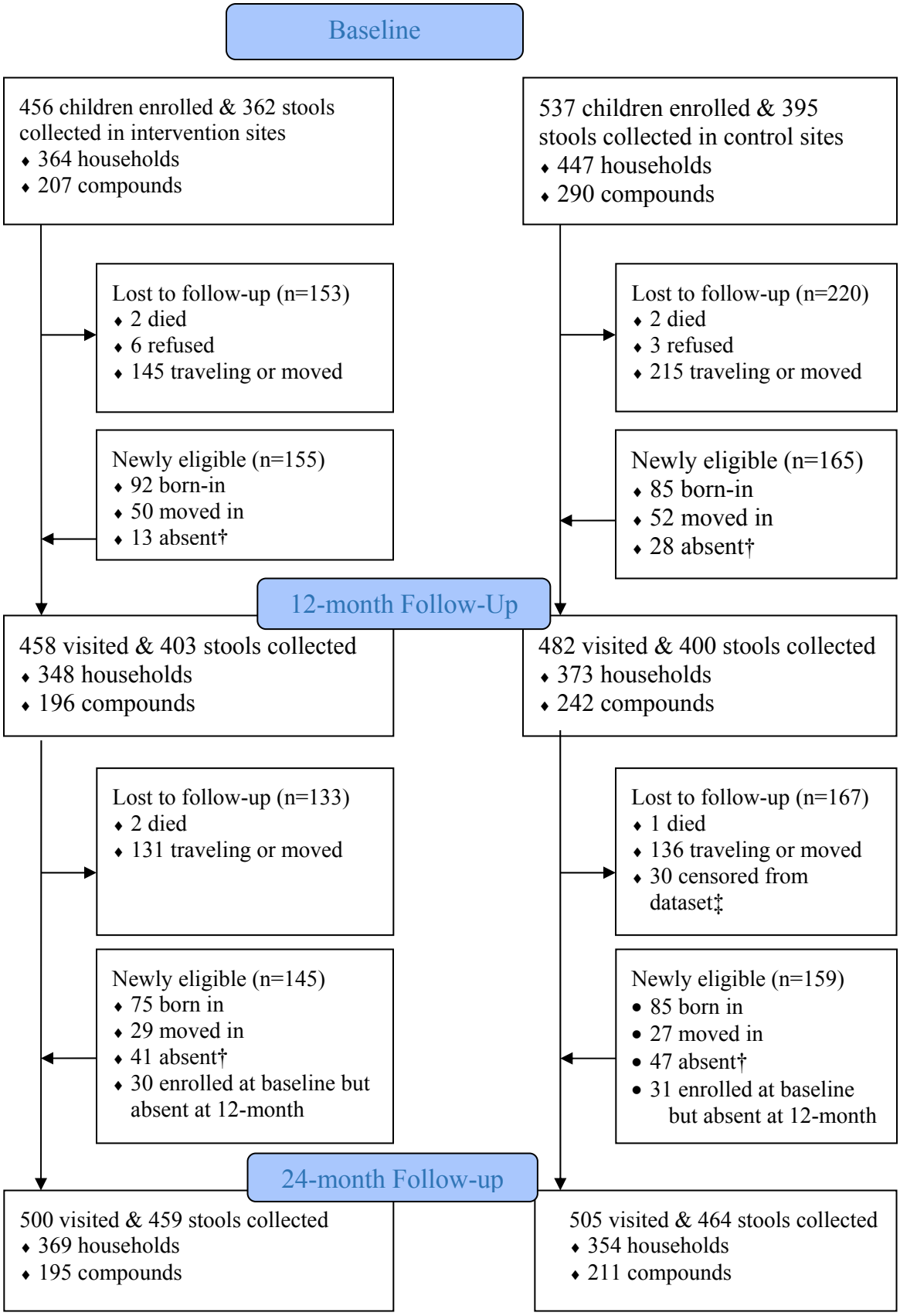
Baseline
456 children enrolled & 362 stools collected in intervention sites
♦ 364 households
♦ 207 compounds
537 children enrolled & 395 stools collected in control sites
♦ 447 households
♦ 290 compounds
Lost to follow-up (n=153)
♦ 2 died
♦ 6 refused
♦ 145 traveling or moved
Lost to follow-up (n=220)
♦ 2 died
♦ 3 refused
♦ 215 traveling or moved
Newly eligible (n=155)
♦ 92 born-in
♦ 50 moved in
♦ 13 absent†
Newly eligible (n=165)
♦ 85 born-in
♦ 52 moved in
♦ 28 absent†
12-month Follow-Up
458 visited & 403 stools collected
♦ 348 households
♦ 196 compounds
482 visited & 400 stools collected
♦ 373 households
♦ 242 compounds
Lost to follow-up (n=133)
♦ 2 died
♦ 131 traveling or moved
Lost to follow-up (n=167)
♦ 1 died
♦ 136 traveling or moved
♦ 30 censored from dataset‡
Newly eligible (n=145)
♦ 75 born in
♦ 29 moved in
♦ 41 absent†
♦ 30 enrolled at baseline but absent at 12-month
Newly eligible (n=159)
• 85 born in
• 27 moved in
• 47 absent†
• 31 enrolled at baseline but absent at 12-month
24-month Follow-up
500 visited & 459 stools collected
♦ 369 households
♦ 195 compounds
505 visited & 464 stools collected
♦ 354 households
♦ 211 compounds

Figure 2: Trial profile. †absent at baseline and missing 12-month/24-month survey - born-in/moved-in status unknown at enrollment. ‡ Children removed from analysis because their compound received an intervention after completion of the baseline phase

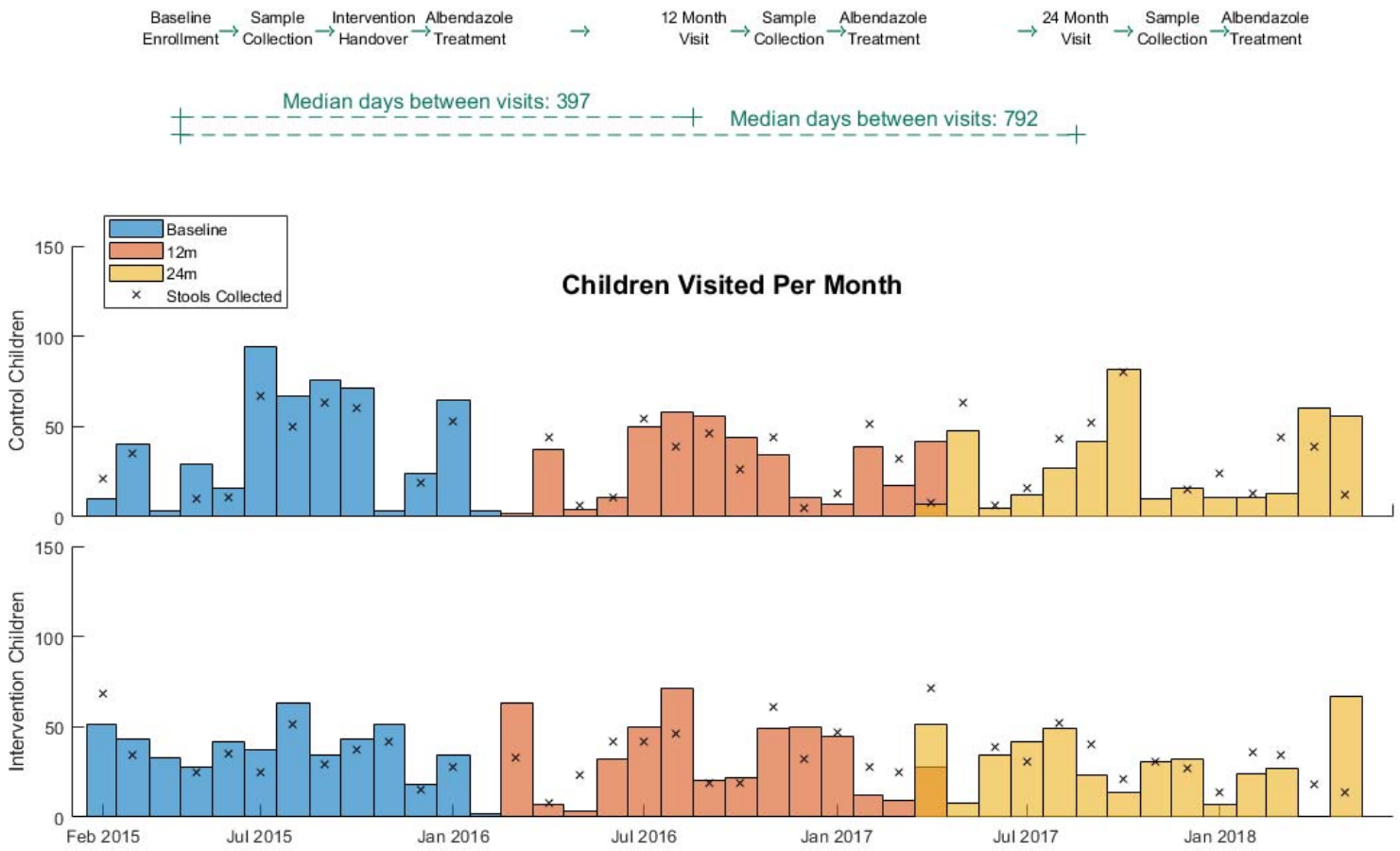

Figure 3: Schematic of study timeline and enrollment and visitation profile for intervention and control children during baseline, 12-month, and 24-month phases.

Of the 155 newly enrolled intervention children, 92 (59%) were born after the baseline visit, 50 (32%) moved in after baseline, and 13 (8.4%) were absent for reasons not captured by the study team. In control compounds, 85/165 (52%) newly enrolled children were born in after baseline, 52 (32%) moved in, and 28 (17%) were absent. The median number of days between baseline enrollment and the 12-month follow-up visit was 397.

At the 24-month visit (April 2017 – September 2018), we visited 500 children in intervention compounds and 505 children in control compounds. We collected stool material from 92% of intervention (464/505) and control (459/500) children and reported diarrhea data from 81% (407/500) of intervention children and 78% (392/505) of control children. Children available for data collection at 24-month included 145 children newly enrolled in the intervention arm (75 born in, 29 moved in, 41 absent at baseline and 12-month visits for unspecified reasons), 159 newly enrolled in the control arm (85 born in, 27 moved in, 47 absent), and 61 children (30 intervention, 31 control) who were enrolled at baseline but absent during the 12-month visit. A total of 300 children (133 intervention, 167 control) available during the 12-month visit were unavailable during the 24-month visit. Of the children originally enrolled during baseline, 233/456 (51.1%) of intervention children and 217/537 (40.4%) of control children were available during all three phases of data collection, and 73% (333/456) and 65% (348/537) of intervention and control children enrolled at baseline, respectively, were available during at least one follow-up phase.

Enrollment and follow-up visitation of intervention and control compounds occurred at a similar rate in each phase (Figure 3). Concurrent enrollment should have limited any

differential effect of seasonality or weather-related secular trends on the outcome in intervention and control arms, as these variables would have affected both arms equally in the aggregate.

Baseline population characteristics and balance

The average age at baseline enrollment was 23 months (SD = 13) and 51% of enrollees were female (Table 5). 32% of children were still breastfeeding at the time of enrollment, including 9% who were exclusively breastfeeding, and 66% wore diapers. A little over half of all caregivers had completed primary school. Measures of all child-level variables were similar among intervention and control children at baseline. All households with an enrolled child used a private municipal water tap as their primary drinking water source at baseline, though it was more common for the tap to be inside compound boundaries in intervention compounds. Households in intervention compounds scored slightly lower on the asset-based wealth index than control households, though the magnitude of difference was small. Control households more frequently had covered floors (concrete, wood, or other) and walls constructed with sturdy materials like bricks, concrete, or wattle and daub. Controls household also had almost one fewer person in residence than intervention compounds though the frequency of household crowding (>3 persons/room) was similar among arms. Differences in household construction and number of residents contributed to the difference observed in household wealth between intervention and control. (Table 5). On average, intervention compounds had higher populations at baseline than control: the mean population of intervention compounds was 19 compared with 15 in controls. In addition to having larger resident populations, intervention sites also had more

households, more water taps, and higher population density (measured in persons/m^2) (Table 5). Both intervention and control sites had an average of one latrine at baseline. Drophole covers and sturdy latrine walls were less common in control compounds than intervention at baseline.

Table 5: Baseline characteristics measured in questionnaires and by direct observation at the child, household, and compound level by study arm

	Control		Intervention		
	n		n		p-value
Child level variables					
Child age, days	524	698 (406)	443	693 (416)	0.84
Child sex, female	524	268 (51%)	445	227 (51%)	0.967
Child is breastfed, with or without complementary feeding	530	171 (32%)	450	145 (32%)	0.989
Child is exclusively breastfed	530	49 (9.3 %)	450	40 (8.9%)	0.847
Child feces disposal,in latrine or potty	530	148 (28%)	450	141 (31%)	0.244
Child wears diapers	530	346 (65%)	450	296 (66%)	0.833
Caregiver completed primary school	530	287 (54%)	450	240 (53%)	0.798
Child's mother is alive	517	507 (98%)	437	428 (98%)	0.89
Respondent is child's mother	523	371 (71%)	445	284 (64%)	0.018
Household level variables					
Household population	439	5.3 (2.3)	357	6.2 (3.0)	<0.001
Household wealth score, 1 (poorer) - 100 (wealthier)†	439	45 (10)	357	44 (11)	0.0306
Household crowding, >3 persons/room	444	54 (12%)	363	60 (17%)	0.076
Household floor is covered‡	444	430 (97%)	363	331 (91%)	0.001

Table 5 (continued).

Household wall made of bricks, concrete, or similar‡	444	305 (69%)	363	215 (59%)	0.005
Household drinking water source inside compound	439	327 (74%)	358	292 (82%)	0.017
Latrine used by household					
Has a ceramic or masonry pedestal‡	436	154 (35%)	357	141 (39%)	0.226
Has a drophole cover‡	438	232 (53%)	357	225 (63%)	0.004
Compound level variables					
Compound population	290	14 (6.2)	207	19 (12)	<0.001
Number of households	275	3.9 (2.1)	207	4.4 (3.7)	0.0652
Number of water taps inside the compound	285	0.97 (0.95)	202	1.4 (1.6)	0.0002
Number of latrines in the compound	285	1.02 (0.21)	202	1.07 (0.61)	0.2536
Latrine walls made of brick, concrete or similar‡	285	72 (25%)	203	68 (33%)	0.047
Compound population density, persons/square meter*	284	0.070 (0.039)	204	0.087 (0.050)	<0.001
Compound has electricity that normally functions	290	252 (87%)	207	189 (91%)	0.126
Compound prone to flooding	290	186 (64%)	207	120 (58%)	0.164
Any animals observed in compound‡	290	171 (59%)	207	132 (63%)	0.279
Dog observed‡	290	14 (4.8%)	207	14 (6.8%)	0.356
Chicken or duck(s) observed‡	290	41 (14%)	207	29 (14%)	0.968
Cats observed‡	290	150 (52%)	207	116 (56%)	0.342

Data are n (%) or mean (SD) and collected by questionnaire unless otherwise noted. †Assessed using Simple Poverty Scorecard for Mozambique, ‡Data collected by direct observation, *Calculated as # of people living in the compound divided by the area of the compound in square meters. Area measurement described previously (Chapter 2).

Balance of baseline population characteristics of children who remained in the study through the 12-month and 24-month visits and children who were lost to follow-up are presented in Tables D1 and D2 (Appendix D). Some characteristics of children lost to follow-up differed from those of children who remained in the study, though differences tended to be consistent in both study arms with few exceptions. Children lost to follow-up by the 12-month visit in the intervention arm were more likely to reside in compounds without chickens, more likely to have a caregiver who had not completed primary school, and lived in compounds with fewer water taps than intervention children who remained in the study. These differences were not observed in control children (Table D1). In comparison, control children were more frequently lost to follow-up from households and compounds with smaller populations and less crowding (Table D1). – a trend observed in both intervention and control children in an analysis of children lost to follow-up by the 24-month phase (Table D2). While rates of enrollment of new participants were similar between study arms at the 12-month and 24-month visits, some variation observed in the characteristics of newly enrolled children compared with children enrolled at baseline was not consistent in both arms (Tables D3 and D4). Compared with differences among new and original enrollees of control compounds, new enrollees in intervention sites at the 24-month visit had slightly higher wealth scores, came from smaller households (population), and were less likely to be a part of compounds with animals than original intervention enrollees (Table D4).

Estimation of intervention effect on all enrolled children (main analysis)

Our main analysis estimated the effect of the sanitation intervention on enteric infection and caregiver reported diarrhea among all enrolled children regardless of phase of enrollment or loss to follow-up prior to study completion. Effect estimates for all analyses are presented as adjusted risk ratios with 95% confidence intervals. Unadjusted risk ratios are also presented for comparison. Confidence intervals of risk ratios which include the null value (1.0) indicate the intervention latrine had no effect on the specified outcome by traditional methods of statistical hypothesis testing.

Enteric infection was common at baseline, and the observed prevalence of our primary outcome (84%, 95% CI: 81 – 87%), any bacterial or protozoan infection, exceeded the figure we used in our sample size calculations (70%) (2,128). The prevalence of most enteric pathogens was similar among intervention and control children at baseline (Table 6). However, the prevalence of the primary outcome was higher among controls (87%) than intervention (81%) children at baseline as was the prevalence of any bacterial infection (79% vs 73%), which is a component of the primary outcome. Coinfection (concurrent infection with two or more measured pathogens) was common (58%) at baseline and detected at a similar frequency in intervention and control children. We detected an average of 1.79 (SD=1.23) pathogens in intervention children and 1.85 (SD=1.17) in control children at baseline. The most frequently detected pathogens at baseline, among both intervention and control, included *Giardia*, *Shigella*, ETEC, *Salmonella*, and norovirus GI/GII. Bacterial infections were the most common type of infection, followed by protozoan, and viral. We did not detect *V. cholerae* in any of the

specimens assayed and detected very low prevalence of *Yersinia pestis*, rotavirus A, *Entamoeba histolytica*, *Cryptosporidium*, and *E. coli* O157. The prevalence of caregiver reported diarrhea was 13% in both intervention and control arms at baseline.

In both study arms, the prevalence of most enteric infections at follow-up phases was similar to or higher than baseline prevalence with few exceptions. The mean number of infections per child and prevalence of coinfection increased with each phase in controls. In intervention children, the mean number of infections and prevalence of coinfection increased between baseline and the 12-month phase but leveled off by the 24-month phase (Table 6). *Giardia*, *Shigella*, ETEC, *Salmonella*, and Norovirus GI/GII remained the most prevalent infections detected in both arms during follow-up phases. The frequency of caregiver-reported diarrhea did not vary substantially across phases in intervention children. In control children, the prevalence of reported diarrhea decreased between baseline and 12-month but returned to baseline levels by the 24-months phase.

Cumulative rainfall in the 30-days prior to sample collection was considered as a potential covariate in the main analysis models to capture the potential effects of seasonality. During model building, we found rainfall to have no meaningful impact on effect estimates and it was dropped from the models in the main analysis and both sub-group analyses.

Table 6: Prevalence (%) of enteric infections and caregiver reported diarrhea in intervention and control children at each study phase

	Baseline		12-month		24-month		Combined Follow-up	
	Control (n=395)	Intervention (n=362)	Control (n=400)	Intervention (n=403)	Control (n=464)	Intervention (n=459)	Control (n=864)	Intervention (n=862)
Primary outcome	87 (83-90)	81 (77-85)	87 (83-90)	88 (84-91)	91 (87-93)	88 (84-90)	89 (86-91)	88 (85-90)
Bacterial	79 (74-83)	73 (69-78)	76 (71-80)	76 (72-80)	80 (76-84)	74 (70-78)	78 (75-81)	75 (72-78)
Shigella	46 (41-51)	42 (37-47)	51 (46-56)	52 (47-57)	59 (54-63)	53 (48-57)	55 (52-58)	52 (49-56)
ETEC	30 (25-35)	30 (25-35)	34 (30-39)	35 (31-40)	27 (23-32)	27 (23-32)	31 (27-34)	31 (28-34)
Salmonella enterica	23 (19-27)	18 (14-23)	17 (13-21)	16 (13-20)	16 (13-20)	14 (11-18)	16 (14-19)	15 (13-18)
Campylobacter	10 (7.3-14)	5.8 (3.6-8.7)	8.0 (5.5-11)	7.9 (5.5-11)	11 (7.9-14)	7.2 (5.0-9.9)	9.4 (7.5-12)	7.5 (5.9-9.5)
C. difficile	5.6 (3.5-8.3)	3.6 (1.9-6.1)	3.5 (1.9-5.8)	3.7 (2.1-6.1)	3.0 (1.7-5)	2.4 (1.2-4.2)	3.2 (2.2-4.6)	3.0 (2.0-4.4)
STEC	1.0 (0.3-2.6)	2.5 (1.1-4.7)	2.0 (0.9-3.9)	1.2 (0.4-2.9)	3.7 (2.1-5.8)	3.1 (1.7-5.1)	2.9 (1.9-4.2)	2.2 (1.3-3.4)
E. coli O157	3.3 (1.8-5.6)	5 (3-7.7)	3.8 (2.1-6.1)	3.5 (1.9-5.8)	5.4 (3.5-7.9)	3.1 (1.7-5.1)	4.6 (3.3-6.3)	3.2 (2.2-4.7)
Yersinia pestis	ND[†]	0.3 (0-1.5)	ND	ND	ND	0.2 (0.0-1.2)	ND	0.1 (0.0-0.6)
Protozoan	52 (47-57)	54 (48-59)	58 (53-63)	62 (57-67)	66 (62-70)	63 (59-68)	62 (59-66)	63 (60-66)
Giardia	51 (46-56)	51 (46-56)	56 (51-61)	60 (55-65)	64 (59-68)	62 (57-66)	60 (57-64)	61 (58-64)
Cryptosporidium	2.0 (0.9-4)	4.4 (2.5-7.1)	2.3 (1-4.2)	3.5 (1.9-5.8)	3.0 (1.7-5.0)	3.3 (1.8-5.3)	2.7 (1.7-4.0)	3.4 (2.3-4.8)
E. histolytica	ND[1]	1.1 (0.3-2.8)	0.5 (0.1-1.8)	1.7 (0.7-3.5)	0.6 (0.1-1.9)	2.2 (1-4)	0.6 (0.2-1.3)	2.0 (1.2-3.1)

Table 6 (continued).

Viral	14 (10-17)	14 (11-18)	14 (10-17)	10 (7.6-14)	13 (9.8-16)	13 (10-17)	13 (11-16)	12 (9.9-14)
norovirus GI/GII	9.9 (7.1-13)	11 (7.8-14)	12 (9-16)	8.4 (5.9-12)	10 (7.5-13)	12 (9.0-15)	11 (9.0-13)	10 (8.3-12)
adenovirus 40/41	3.3 (1.8-5.6)	2.5 (1.1-4.7)	1.8 (0.7-3.6)	2.2 (1-4.2)	1.5 (0.6-3.1)	1.3 (0.5-2.8)	1.6 (0.9-2.7)	1.7 (1-2.9)
rotavirus A	0.8 (0.2-2.2)	1.9 (0.8-3.9)	ND	0.2 (0-1.4)	1.1 (0.4-2.5)	0.4 (0.1-1.6)	0.6 (0.2-1.3)	0.3 (0.1-1)
Coinfection	60 (55-65)	57 (51-62)	63 (58-68)	65 (60-70)	70 (66-74)	64 (59-68)	67 (64-70)	64 (61-68)
Number of infections‡	1.85 (1.17)	1.79 (1.23)	1.92 (1.16)	1.97 (1.16)	2.05 (1.16)	1.91 (1.13)	1.99 (1.16)	1.94 (1.15)
Reported diarrhea	13 (9.9-16) n=530	13 (10-17) n=450	9.2 (6.7-12) n=435	14 (11-17) n=432	14 (10-17) n=392	13 (9.9-17) n=407	11 (9.2-14) n=827	13 (11-16) n=839

Data presented as prevalence (%) and 95% CI unless noted. n= # of unique children. *V. cholerae* not detected at any phase. † Not detected , ‡ mean (SD)

The intervention had no effect on the prevalence of the primary outcome in adjusted or unadjusted analyses at the 12-month follow-up phase (aRR 1.08, 95% CI: 0.99-1.18). The intervention also had no measurable effect at 12-months on risk of infection with any of the three pathogen types (bacterial, protozoan, viral) or on any individual pathogen tested by the GPP (Table 7). While the results of the analysis of protozoan pathogens were driven by a single, highly prevalent pathogen, *Giardia*, numerous bacterial pathogens contributed to results of the bacterial analysis, with all targets but *V. cholerae* detected, and four bacterial pathogens detected in >5% of samples. The intervention did not affect the risk of coinfection. The results of the 12-month bacterial and protozoan analyses were similar in magnitude and direction to one another. Conversely, the measure of effect for viral targets, though not significant, went in the opposite direction (RR, aRR <1) of the bacterial and protozoan measures (RR, aRR>1). The results of adjusted analyses were largely consistent with unadjusted results at 12-month.

At 24-months, the intervention had no effect on the primary outcome, or any of the individual pathogens or pathogen types. For most pathogen outcomes, estimates of intervention effect at 24-months decreased in magnitude and were subsequently closer to the null value (RR, aRR = 1) than at the 12-month analysis. In some instances, the direction of the effect estimate varied between the 12-month and 24-month phases (Table 7).

Results from the combined follow-up analysis (including observations from both the 12-month and 24-month phases) were largely consistent with results from the individual 12- and 24-month analyses. We did not observe the intervention to have a measurable effect

on the risk of the primary outcome, any pathogen group, or any individual pathogen target (Table 7). Results from adjusted and unadjusted analyses were similar. We could not calculate effect estimates at any phase for *Y. pestis*, *E. histolytica*, or rotavirus due to sparse data.

The intervention had no measurable effect on the risk of reported diarrhea after 12-months or 24-months of intervention exposure when phase results were analyzed separately or combined into a single follow-up phase. The adjusted and unadjusted analyses yielded similar results. As observed with the pathogen results, the magnitude of the effect estimate was smaller at 24-months than at 12-months and changed direction.

Table 7: Effect of intervention on enteric infection and reported diarrhea in all enrolled children at 12-months, and 24-months and with follow-up data combined.

	12-month		24-month		Follow-up combined	
	RR, n=1122 (1560)	aRR, n=1071 (1454)	RR, n=1321 (1680)	aRR, n=1135 (1412)	RR, n=1474 (2483)	aRR, n=1320 (2138)
Primary outcome	1.08 (0.99-1.17)	1.08 (0.99-1.18)	1.03 (0.95-1.11)	1.02 (0.94-1.11)	1.05 (0.98-1.13)	1.05 (0.97-1.13)
Bacterial	1.08 (0.96-1.21)	1.09 (0.97-1.23)	1.00 (0.89-1.11)	0.99 (0.87-1.12)	1.03 (0.94-1.14)	1.04 (0.94-1.16)
Shigella	1.12 (0.91-1.39)	1.14 (0.94-1.39)	0.97 (0.79-1.19)	0.96 (0.79-1.16)	1.04 (0.86-1.26)	1.04 (0.88-1.24)
ETEC	1.02 (0.74-1.41)	1.05 (0.75-1.46)	0.98 (0.69-1.39)	0.87 (0.60-1.26)	1.00 (0.74-1.36)	0.96 (0.71-1.32)
Salmonella	1.18 (0.77-1.81)	1.22 (0.79-1.89)	1.11 (0.72-1.71)	1.18 (0.74-1.88)	1.15 (0.80-1.65)	1.22 (0.84-1.77)
Campylobacter	1.70 (0.84-3.45)	1.52 (0.72-3.21)	1.18 (0.58-2.38)	1.23 (0.56-2.70)	1.38 (0.73-2.60)	1.34 (0.68-2.65)
C. difficile	1.64 (0.60-4.50)	1.76 (0.64-4.88)	1.24 (0.44-3.54)	1.49 (0.49-4.57)	1.43 (0.62-3.33)	1.63 (0.69-3.85)
STEC	0.25 (0.05-1.11)	0.25 (0.06-1.13)	0.33 (0.08-1.35)	0.28 (0.07-1.13)	0.30 (0.08-1.10)	0.28 (0.08-1.00)
E. coli O157	0.62 (0.23-1.65)	0.60 (0.23-1.54)	0.39 (0.13-1.17)	0.62 (0.19-2.05)	0.47 (0.19-1.18)	0.60 (0.24-1.49)
Yersinia	-[†]	-	-	-	-	-
Protozoan	1.06 (0.88-1.27)	1.03 (0.87-1.23)	0.92 (0.77-1.10)	0.93 (0.78-1.12)	0.98 (0.83-1.15)	0.98 (0.84-1.15)
Giardia	1.08 (0.90-1.30)	1.06 (0.89-1.26)	0.95 (0.80-1.14)	0.97 (0.82-1.16)	1.01 (0.85-1.19)	1.02 (0.87-1.19)

Table 7 (continued).

Cryptosporidium	0.75 (0.19-2.86)	0.87 (0.22-3.35)	0.47 (0.11-1.95)	0.47 (0.10-2.15)	0.54 (0.17-1.75)	0.59 (0.18-1.96)
E. histolytica	-	-	-	-	-	-
Viral	0.71 (0.41-1.22)	0.68 (0.39-1.15)	0.97 (0.55-1.70)	1.07 (0.57-1.98)	0.84 (0.52-1.35)	0.86 (0.53-1.39)
Norovirus GI/GII	0.62 (0.33-1.16)	0.57 (0.30-1.06)	1.02 (0.53-1.96)	1.05 (0.52-2.14)	0.81 (0.46-1.42)	0.78 (0.44-1.39)
Adenovirus 40/41	1.79 (0.42-7.63)	1.58 (0.38-6.57)	1.19 (0.24-6.02)	1.38 (0.22-8.83)	1.49 (0.42-5.34)	1.54 (0.42-5.64)
Rotavirus A	-	-	-	-	-	-
Coinfection	1.10 (0.94-1.28)	1.09 (0.94-1.28)	0.96 (0.83-1.12)	0.97 (0.83-1.14)	1.02 (0.89-1.17)	1.03 (0.90-1.19)
Number of infections	1.06 (0.93-1.20)	1.06 (0.94-1.19)	0.94 (0.83-1.07)	0.95 (0.84-1.08)	0.99 (0.89-1.11)	1.01 (0.90-1.12)
Reported diarrhea	1.43 (0.81-2.53) n=1287 (1847)	1.36 (0.77-2.39) n=1253 (1812)	0.93 (0.56-1.56) n=1358 (1779)	0.93 (0.55-1.57) n=1318 (1773)	1.15 (0.73-1.82) n=1516 (2646)	1.13 (0.71-1.8) n=1471 (2594)

Results presented as risk ratios (95% CI) unless specified, n= # unique children included in analysis (# unique stools included in analysis or # of unique data points in the case of reported diarrhea), *V. cholerae* not detected in any samples and excluded from analysis, †Could not be calculated due to sparse data

Sub-group analysis: impact of intervention on children available at 2 (or more) phases

We performed a sub-group analysis to evaluate the impact of the intervention on children who were enrolled during the baseline phase, remained in the study through the 12-month or 24-month phase, and provided a stool specimen (or reported diarrhea data) at baseline and at least one follow-up phase. The sample sizes for these analyses were 61-75% smaller than the comparable main analyses given the additional inclusion restrictions and high rate of loss to follow-up observed in our study population.

The results of these sub-group analyses were consistent with results from the main analyses: we found no evidence of an effect of the intervention on the prevalence of the primary outcome after 12-months of exposure or after 24-months of exposure (Table 8). We also found no effect of the intervention on the primary outcome when we combined observations from the 12-month and 24-month phases. Results for pathogen groups and individual pathogens were largely similar to results from the main analysis and demonstrated no measurable intervention effects. Effect estimates for *Campylobacter*, *C. difficile*, STEC, *E. coli* O157, *Cryptosporidium*, and adenovirus 40/41 could not be precisely estimated due to low prevalence coupled with the reduced samples size included in the sub-group analysis. Similar to results from the main analysis, we observed the magnitude of RR and aRRs were often smaller in the 24-month analysis than the 12-month analysis. We also found no impact of the intervention on the occurrence of caregiver-reported diarrhea at 12-months, 24-months, or the combined 12- and 24-month follow-up analysis.

Table 8: Effect of intervention on enteric infection and reported diarrhea in children with stools or reported diarrhea data collected at 2 or more phases (including baseline) at 12-months, and 24-months and combined follow-up data.

	12-month		24-month		Follow-up combined	
	RR, n=438 (876)	aRR, n=383 (766)	RR, n=359 (718)	aRR, n=277 (554)	RR, n=498 (1295)	aRR, n=414 (1034)
Primary outcome	1.08 (0.97-1.19)	1.07 (0.96-1.19)	1.02 (0.92-1.12)	1.03 (0.92-1.15)	1.05 (0.97-1.15)	1.08 (0.98-1.19)
Bacterial	1.04 (0.90-1.20)	1.06 (0.91-1.23)	0.99 (0.86-1.13)	1.00 (0.85-1.16)	1.01 (0.90-1.14)	1.06 (0.93-1.21)
Shigella	1.09 (0.86-1.38)	1.15 (0.90-1.46)	0.94 (0.72-1.23)	1.12 (0.83-1.50)	1.03 (0.84-1.28)	1.15 (0.92-1.45)
ETEC	0.98 (0.65-1.47)	1.02 (0.66-1.57)	0.91 (0.55-1.49)	0.94 (0.55-1.61)	0.94 (0.64-1.38)	1.04 (0.70-1.56)
Salmonella	1.59 (0.88-2.90)	1.55 (0.83-2.91)	1.16 (0.54-2.48)	1.21 (0.51-2.86)	1.35 (0.80-2.25)	1.45 (0.84-2.51)
Campylobacter‡	1.37 (0.52-3.60)	1.20 (0.41-3.52)	1.34 (0.50-3.57)	1.72 (0.61-4.88)	1.40 (0.61-3.20)	1.37 (0.55-3.43)
C. difficile‡	1.88 (0.32-11.1)	2.28 (0.28-18.4)	0.28 (0.03-2.95)	0.19 (0.02-2.24)	1.00 (0.23-4.44)	1.18 (0.23-6.04)
STEC‡	0.27 (0.03-2.11)	0.35 (0.04-2.83)	0.85 (0.08-8.83)	2.29 (0.15-35.0)	0.44 (0.09-2.22)	0.43 (0.07-2.8)
E. coli O157‡	0.84 (0.28-2.53)	1.95 (0.46-8.30)	0.69 (0.14-3.4)	0.80 (0.14-4.51)	0.59 (0.20-1.72)	1.37 (0.39-4.79)
Yersinia †	-	-	-	-	-	-
Protozoan	1.18 (0.94-1.48)	1.17 (0.94-1.47)	0.94 (0.74-1.2)	1.02 (0.79-1.31)	1.1 (0.90-1.34)	1.13 (0.92-1.39)
Giardia	1.16 (0.92-1.46)	1.15 (0.92-1.45)	0.94 (0.74-1.19)	1.04 (0.81-1.33)	1.09 (0.89-1.34)	1.12 (0.90-1.39)

Table 8 (continued).

Cryptosporidium[1]	1.75 (0.26-11.6)	3.50 (0.33-37.28)	0.57 (0.06-5.38)	0.24 (0.02-2.81)	0.99 (0.20-4.83)	1.29 (0.26-6.40)
E. histolytica †	-	-	-	-	-	-
Viral	0.79 (0.39-1.63)	0.82 (0.39-1.76)	0.89 (0.39-2.02)	0.69 (0.27-1.74)	0.76 (0.41-1.41)	0.75 (0.39-1.46)
Norovirus GI/GII	0.61 (0.27-1.39)	0.68 (0.28-1.62)	0.68 (0.27-1.74)	0.41 (0.14-1.22)	0.60 (0.29-1.22)	0.55 (0.25-1.22)
Adenovirus 40/41[1]	3.56 (0.46-27.3)	2.23 (0.27-18.5)	6.11 (0.48-78.2)	5.15 (0.38-69.3)	3.71 (0.62-22.2)	3.18 (0.56-18.1)
Rotavirus A †	-	-	-	-	-	-
Coinfection	1.15 (0.94-1.40)	1.15 (0.94-1.39)	0.98 (0.80-1.19)	1.03 (0.84-1.27)	1.07 (0.90-1.26)	1.13 (0.95-1.35)
Number of infections	1.13 (0.97-1.31)	1.15 (0.99-1.34)	0.97 (0.82-1.14)	1.01 (0.84-1.21)	1.05 (0.91-1.2)	1.14 (0.99-1.31)
Reported diarrhea	1.71 (0.78-3.78) n= 560 (1120)	1.78 (0.82-3.90) n=559 (1118)	0.76 (0.35-1.65) n=421 (842)	0.73 (0.33-1.58) n=415 (830)	1.18 (0.64-2.18) n=643 (1624)	1.21 (0.63-2.34) n=562 (1460)

Results presented as risk ratios (95% CI) unless specified. n= # of unique children (# of unique samples assayed), *V. cholerae* not detected in any samples and excluded from analysis. ‡Effect estimate(s) imprecise due to low prevalence, † Could not be calculated due to sparse data

Sub-group analysis: impact of the intervention on children born into the study

To assess the effect of the intervention on children who had spent their entire lives exposed to the intervention, we performed a sub-group analysis which compared the prevalence of infection and reported diarrhea in children born into study compounds after the baseline visit (controls) or after the intervention latrine was opened for use with children of a similar age at baseline. For example, children born into the study population after the baseline visit and less than one year old at the time of the 12-month visit were compared with children who were less than one year of age at baseline; children born into the study and less than two years old by the 24-month visit were compared with children less than two years old at baseline. Because the analysis inclusion criteria drastically reduced the available sample size, we could not precisely model effect estimates for individual pathogen targets in the 12-month analysis. Effect estimates for pathogens with low prevalence (*Campylobacter*, *C. difficile,* STEC, *E. coli* O157, *Cryptosporidium*, and adenovirus 40/41) remained imprecise in the 24-month and combined analyses.

The 12-month analysis included 103 (55 intervention, 48 control) children born into the study after baseline and 211 (100 intervention, 111 control) children of a comparable age at baseline (<1 year old). The intervention did not have a measurable effect on any of the infection outcomes in children born into the study before the 12-month visit (Table 9). The confidence intervals of our effect estimates were wide due to the small sample size and may have masked our ability to detect an effect.

Table 9: Effect of intervention on enteric infection and reported diarrhea in children born after baseline (compared to children of similar age at baseline) at 12-months, and 24-months and combined follow-up data.

	12-month		24-month		Follow-up combined	
	RR, n=314 (314)	aRR, n=308 (308)	RR, n=647 (647)	aRR, n=619 (619)	RR, n=691 (750)	aRR, n=673 (722)
Primary outcome	1.16 (0.84-1.62)	1.29 (0.94-1.79)	1.06 (0.88-1.28)	1.06 (0.88-1.29)	1.09 (0.90-1.3)	1.09 (0.91-1.31)
Bacterial	1.26 (0.87-1.82)	1.38 (0.96-2)	0.97 (0.76-1.23)	0.98 (0.77-1.27)	1.05 (0.85-1.31)	1.08 (0.86-1.35)
Shigella	1.22 (0.32-4.69)	1.38 (0.36-5.3)	0.49 (0.28-0.86)	0.53 (0.29-0.95)	0.62 (0.37-1.03)	0.69 (0.41-1.16)
ETEC	0.95 (0.39-2.27)	1.03 (0.42-2.51)	0.92 (0.51-1.67)	0.86 (0.48-1.55)	0.93 (0.54-1.61)	0.89 (0.52-1.51)
Salmonella	1.47 (0.74-2.92)	1.64 (0.81-3.35)	1.50 (0.81-2.76)	1.67 (0.90-3.10)	1.43 (0.86-2.37)	1.52 (0.91-2.53)
Campylobacter ‡	2.14 (0.46-10)	3.13 (0.67-14.5)	1.94 (0.69-5.46)	1.97 (0.69-5.66)	1.88 (0.73-4.80)	1.88 (0.72-4.91)
C. difficile‡	1.36 (0.42-4.42)	1.42 (0.44-4.52)	1.30 (0.36-4.78)	1.43 (0.39-5.23)	1.47 (0.57-3.77)	1.45 (0.56-3.73)
STEC ‡	-†	-†	0.11 (0.01-1.79)	0.10 (0.01-1.66)	0.20 (0.02-2.18)	0.19 (0.02-2.17)
E. coli O157‡	7.37 (0.42-130)	-†	0.44 (0.05-3.61)	0.49 (0.06-3.9)	0.43 (0.05-3.51)	0.44 (0.06-3.51)
Yersinia†	-	-	-	-	-	-
Protozoan	0.41 (0.12-1.43)	0.57 (0.16-2.03)	0.85 (0.56-1.30)	0.88 (0.59-1.32)	0.82 (0.54-1.24)	0.86 (0.57-1.29)
Giardia	0.50 (0.14-1.82)	0.72 (0.19-2.71)	0.85 (0.56-1.30)	0.97 (0.64-1.46)	0.91 (0.59-1.39)	0.96 (0.64-1.44)

Table 9 (continued).

Cryptosporidium‡	0.12 (0.01-2.5)	0.24 (0.01-5.26)	0.44 (0.08-2.53) [1]	0.45 (0.08-2.53) [1]	0.40 (0.08-1.95)	0.40 (0.08-1.99) [1]
E. histolytica†	-	-	-	-	-	-
Viral	0.42 (0.15-1.17)	0.45 (0.16-1.22)	0.89 (0.40-1.94)	0.91 (0.41-2.03)	0.72 (0.38-1.38)	0.72 (0.38-1.38)
Norovirus GI/GII‡	0.59 (0.19-1.9)	0.57 (0.18-1.81)	1.34 (0.53-3.41)	1.30 (0.51-3.31)	1.07 (0.49-2.35)	1.03 (0.47-2.25)
Adenovirus 40/41‡	0.56 (0.06-5.03)	0.84 (0.08-8.75)	-†	-†	0.20 (0.03-1.32)	0.17 (0.02-1.18)
Rotavirus A†	-	-	-	-	-	-
Coinfection	0.83 (0.46-1.51)	1.04 (0.59-1.84)	0.86 (0.61-1.22)	0.88 (0.62-1.25)	0.89 (0.65-1.22)	0.91 (0.66-1.25)
Number of infections	0.90 (0.61-1.32)	1.04 (0.71-1.5)	0.91 (0.7-1.17)	0.93 (0.72-1.19)	0.91 (0.73-1.14)	0.93 (0.75-1.16)
Reported diarrhea	0.33 (-0.74-1.41) n=382 (382)	1.51 (0.53-4.3) n=377 (377)	1.20 (0.57-2.55) n=733 (733)	1.33 (0.60-2.96) n=716 (716)	1.26 (0.65-2.43) n=798 (852)	1.37 (0.69-2.72) n=781 (835)

Results presented as risk ratios (95% CI) unless specified, n=# of unique children (# of unique stools or reported diarrhea data points), *V. cholerae* not detected in any samples and excluded from analysis, ‡Effect estimate(s) imprecise due to low prevalence, † Could not be calculated due to sparse data

The sample size for the 24-month sub-group analysis was larger than the sample size in the 12-month analysis and included 215 (108 intervention, 107 control) children who had been born into the study after the intervention was open for use (or after the baseline visit in case of controls) but before the 24-months visit and 432 (201 intervention, 231 control) children in the baseline comparison group (<2 years old at 24-month visit). The intervention had no effect on the primary outcome in children <2 years old who had been born into a compound with an intervention latrine but did reduce the risk of *Shigella* infection by almost 50% (aRR 0.53, 95% CI: 0.29-0.95) (Table 9). The intervention had no other measurable effects on infection outcomes in the 24-month sub-group analysis.

We also observed no effect of the intervention on infection outcomes in the 12- and 24-month combined analysis. The intervention had no effect on caregiver reported diarrhea in the 12-month, 24-month, or combined analysis (Table 9).

DISCUSSION

We found little evidence that the intervention, hygienic privately shared pour-flush latrines to septic tanks, reduced the risk of enteric infection or caregiver reported diarrhea in children less than 6 years old (<4 at baseline + 24-month of follow-up) living in the low-income, unplanned neighborhoods of Maputo, Mozambique. Evidence of the effect of sanitation on health is mixed and may be influenced by study setting, study design, intervention infrastructure, and choice of outcome measure among other factors. Findings from previous studies of urban sanitation improvements have largely demonstrated health gains (26,115–117). A non-randomized before-and-after study (without a concurrent control group) of health benefits related to the expansion of the sewerage network in Salvador, Brazil demonstrated reductions in reported-diarrhea (21% reduction) and in the prevalence of objective health outcomes like *Giardia*, *T. trichiura*, and *Ascaris*, which were all reduced by 50 – 72% (115,117). These results aren't anomalous within the urban sanitation literature: two meta-analyses have found that connections to sewerage can result in diarrhea reductions of 25-60% depending on starting conditions (26,31). There are many reasons our findings are inconsistent with much of the urban sanitation literature to date, with the most important being the type of sanitation technology evaluated: MapSan is the *first* health impact evaluation of an on-site sanitation system in an urban setting. Evidence from recent large-scale, rigorous health impact trails of on-site sanitation interventions in rural areas is mixed with most trials demonstrating modest effects or no effects on primary health outcomes (32–37). Recent sanitation trials have

focused on using linear growth (32,33,37)– a more distal measure than enteric infection on the causal chain from exposure to outcome – or reported diarrhea as the primary health outcome (32–36). Only two studies found the sanitation intervention to have a measurable effect on a reported diarrhea (33) or linear growth (36). A subset of these studies have also published results of enteric infection as secondary outcomes and the evidence demonstrates no clear trend across study sites. A trial of combined and independent effects of WASH interventions (including on-site sanitation) in rural Bangladesh, the same trial that found reductions in diarrhea due to the intervention, found that the sanitation intervention reduced prevalence of *Giardia* and soil-transmitted helminth (STH) *T. trichiura* by 25% and 29%, respectively (38,39) but its sister study in Kenya only found reductions in *Ascaris* (and no other pathogens) when sanitation was a component of broader combined WASH intervention (40). A similar study performed in Zimbabwe found a combined WASH and nutrition intervention reduced the prevalence of *Giardia* by 10 percentage points, but the effect was absent in the WASH-only intervention arm (41). Two large scale sanitation trials in India found no effect on infection with *Giardia*, *Cryptosporidium*, *E. histolytica*, or on several STH (34,35). The mixed nature of the evidence suggests that measurable health benefits cannot be assumed, at least in the short term, following sanitation improvements and that the health effect of sanitation interventions is likely highly context specific and dependent on baseline levels of fecal contamination, sanitation coverage, the burden and types of endemic enteric infection, pathways of transmission, setting specific WASH behaviors and practices, and choice of sanitation technology (42).

While we found no effect of the intervention on our pre-specified primary outcome, the intervention substantially reduced the risk of *Shigella* infection in children born into the study by the 24-month follow-up visit. Studying children born into intervention sites after the intervention was implemented allowed us to isolate the effect of the intervention on a naïve gut during the critical first two years of life. While exploratory, this result may suggest that the intervention delays exposure and the accumulation of enteric infection during early childhood. It is important to note that this, and other sub-group analyses of individual pathogens, were exploratory in nature and as such we did not correct for multiple comparisons. Therefore, it is possible that the effect observed on *Shigella* is specious and due to type I error.

We were unable to estimate the effect of the intervention on several enteric pathogens due to their low prevalence. Of note, rotavirus A was detected too infrequently (<1% of samples) to be included in individual pathogen analyses. This result is surprising given that rotavirus has been implicated as one of the main etiologic agents of diarrheal disease in young children in LMICs (43), even in settings as geographically close to our study as the nearby rural province of Manhiça, Mozambique (44). It is unlikely that our low detection frequencies were a result of the rotavirus vaccine, as it was not added to the national immunization schedule of Mozambique until September 2015, approximately half way through our baseline phase. Therefore, we would only expect a subset of study population, specifically those born around or after September 2015, to be affected. It is also unlikely that our results are due to measurement error as we verified a subset (8 positive for rotavius, 84 negative rotavirus as determined by GPP) using an in-vitro

diagnostic ELISA assay (Premier Rotaclone, Meridian Bioscience, Cincinnati, OH, USA). The observed variation in prevalence may suggest substantial variability in endemicity even across short distances.

There are several reasons we may not have detected an effect of the intervention on enteric infection or self-reported diarrhea. The trial was originally powered to detect an effect based on a prospective longitudinal cohort design. One advantage of such designs is increased statistical efficiency that results from taking multiple measurements on the same participants over time. While we exceeded the original enrollment numbers detailed in our sample size calculations (345 per arm) because of progressive enrollment throughout the study, due to the greater than expected losses to follow-up we were not able to follow all children enrolled at baseline through time as expected. The potential reduction in statistical efficiency resulting from losses to follow-up and the subsequent necessity to treat measurements as repeated cross-sectional may have masked our ability to detect small effects. We simulated the original analysis, albeit with a smaller sample size than originally expected, by running a sub-group analysis that analyzed only children with data and stool collected at two or more time-points (including baseline). Results from this sub-group analysis demonstrated no intervention effect on any health outcome.

We do not expect that our null effect finding was driven by a lack of use because the original pre-intervention latrine was removed, and we expect there to be minimal behavior change required to shift from using latrines in poor condition to the intervention latrines. Forthcoming research on the intervention fidelity and use will formally address this question. It is possible that in this setting, where fecal contamination was pervasive

and infection burden was high, that even considerable reductions in contamination and exposure due to the intervention would not have been sufficient to realize measurable health gains (118). Further, the intervention may not have addressed all important compound-level pathways of enteric pathogen transmission in this setting (7). The intervention did not specifically address of child feces disposal practices – which can be an important source of contamination, exposure, and health risk (113,129–132)- and it is unlikely the intervention infrastructure would have changed these behaviors and practices. The intervention would not have limited exposures occurring via consumption of contaminated food, which has been demonstrated as a dominant transmission route for enteric pathogens in some settings (7). Finally, the intervention did not address potential exposure to zoonotic pathogens present in animal feces. The frequent interaction of children with animal feces has been documented in similar settings and could be an important, unmitigated source of exposure to enteric pathogens in both intervention and control arms where animals were frequently observed (133,134).

We hypothesized that the majority of exposures young children experienced in this setting occurred at the compound level (135,136) as that is where young children spent the majority of their time and compounds were typically sounded with walls – limiting exploratory behavior outside of the compound. However, exposures occurring outside of the compound could have influenced health outcomes. The transience of the study population meant that familial trips back and forth to provinces outside of Maputo, where exposures were varied and unmeasured, were common. Additionally, as allocation of the intervention was done at the compound level and intervention sites were interspersed

throughout the 11 study neighborhoods, it's possible that sources of contamination originating outside of an intervention compound's boundaries, such as a neighbor's overflowing pit latrine, could have impacted study compounds and nullified any health gain triggered by the intervention. Communities may need to reach a certain level of sanitation coverage before real health gains are achieved (137). Future spatial analyses of this data will examine the role of sanitation coverage and "spill-over" contamination from the surrounding environment.

Strengths and limitations

There are several important limitations of this study. First, as the intervention was pre-planned by an independent non-governmental organization, WSUP, we could not randomize its allocation and therefore could not use a randomized controlled trial, the gold standard study design for drawing causal inferences, to evaluate the intervention effect. Because of this, the differences we observed between arms at baseline cannot be attributed to randomness and must be evaluated as potential confounders. While none of the variables that were imbalanced between intervention and control at baseline met our criteria for inclusion as covariates in multivariable models, our study has a higher risk of being affected by residual confounding bias (innate, unmeasured differences between the intervention and control groups) than in a randomized design. To address this, we use a difference-in-difference regression analysis which accounts for baseline values of our outcome measures. Adjusting for baseline outcome measurements is often the most effective method of controlling for potential confounding caused by baseline imbalance between the study arms (68,138). One of the key assumptions to ensure the validity of

DID analysis is the parallel trend assumption which asserts that any trend in outcome measures between intervention and control arms should be constant in the absence of the intervention or treatment. While this assumption cannot be proven, we can assess its plausibility using "placebo tests" whereby we rerun analyses using a variable expected to be unaffected by the intervention as the outcome metric (139). Any such analysis should show no effect of the intervention on the chosen outcome. For example, using the sex of enrolled children as the study outcome demonstrates that the intervention has no effect on the proportion of children recorded as female. Similarly, the intervention does not affect the proportion of respondents who report being the mother of an enrolled child when tested as a placebo outcome. We find similar results when using several different variables as placebo study outcomes, strengthening the plausibility of the parallel trend assumption and the validity of the DID approach in general. Because we did not collect multiple pre-intervention data points, we are unable to further assess the parallel trend assumption by comparing pre-intervention trends in study outcome metrics.

Second, while it was not possible to mask participants as to their intervention status, our use of an objectively measured health metric, enteric infection, as our primary study outcome eliminated the risk of courtesy and recall bias in our main outcome measure (118,140). Both types of respondent bias posed threats to the validity of our reported diarrhea outcome measure. To reduce the risk of courtesy bias, our team of field enumerators was different from WSUP's implementation team, and respondents were not informed explicitly that the MapSan study team was evaluating the health effect of the WSUP sanitation intervention. To limit the effect of recall bias, we used a 7-day recall

period, which has been demonstrated as superior, for this reason, to 14-day measures (70).

Third, because of the higher than expected turnover rates in our study population, we modified our original analysis plan and treated our data as repeated cross-sectional measurements. We performed the originally planned prospective cohort analysis as a sub-group analysis. This change may have affected the statistical efficiency of our analysis (141). The population movement into and out of the cohort also could have introduced bias into our effect estimates if it differentially affected the control and intervention arms in a meaningful way. Comparisons of characteristics of children who were present for multiple phases of data collection with children lost to follow-up and children enrolled after baseline demonstrated some degree of differential (non) response bias between study arms. Our two time-point sub-group analysis, which also served as a simulation of pre-specified analysis plan, provided the basis for a sensitivity analysis to estimate the impact of population churn on our effect estimates. Results from the main analysis and the sub-group analysis were similar making it less likely that population churn affected the study results. An additional exploratory analysis that uses inverse probability weighting techniques to adjust for imbalances caused by changes to the study population may be advisable to further interrogate the impact these changes could have on our effect estimates (141).

Fourth, while molecular detection of enteric pathogens in stool is evidence that a child was, at some point, exposed to that pathogen, it does not necessarily indicate active infection, complicating the use of pathogen detection as a health indicator. Pathogen

detection could represent previous symptomatic or asymptomatic infection, pathogen carriage or colonization, or simply passage of the pathogen through the gastrointestinal tract following exposure. However, the GPP was designed specifically to aid in diagnosis of enteric infection and its relatively high limits of detection exceed estimated infectious doses of most pathogens, increasing the likelihood that pathogen detections in this study represent past or present active infections. Because of the high limits of detection, it is important to note that absence of detection does not indicate absence of exposure. While enteric infection is a more proximate outcome to fecal contamination exposure than many other commonly measured health outcomes in WASH impact trials, such as growth, its clinical significance is less clear. Recent studies, including this one, have observed high levels of asymptomatic infection, or infection which is not accompanied by typical symptoms of diarrheal disease (43,46,128). Further, our repeated cross-sectional qualitative measures of enteric infection do not provide information on the duration or intensity of infection, making it unclear whether repeated detection of a pathogen represented clearance and re-infection or persistent infection or carriage. *Giardia*, one of the most common pathogens we detected at baseline and follow-up phases, can cause persistent infection lasting months (101,142). The GPP detects 15 enteric pathogens, including many implicated as the main causes of childhood diarrhea in LMICs in recent studies (43,46), however, it does not detect all enteric pathogens of potential importance and our results cannot be considered a comprehensive accounting of infection burden. Further, while several studies have shown good specificity of the GPP for detection of most targets included in the assay (76,78,79,82,84,143), there have been a few recent studies that demonstrate higher than expected false positive detection rate for the

Salmonella molecular targets (80,144). In response to these studies, Luminex, the manufacturer of the GPP, has recently amended the criterion (median fluorescence intensity thresholds) for determining *Salmonella* detections. The results presented here use the original version of the criterion and a future reanalysis of results that uses the updated criterion or excludes *Salmonella* from the primary outcome definition would be prudent.

Fifth, we had limited ability to comprehensively evaluate the impact of seasonality or weather-related trends, which have demonstrated associations with diarrheal disease risk in the literature (145,146), on our effect estimates. Mozambique typically experiences a rainy season lasting from November and March during which time flooding can be common, especially in the low-income, densely populated urban neighborhoods where drainage infrastructure is poor. However, rainfall during the 2015/2016 rainy season, which occurred during baseline data collection, was far below average leading to widespread drought (147,148). Because of this, we were unable to fully evaluate whether the effect of the intervention may be modified by the rainfall or flooding typical of a rainy season in Mozambique. We did assess cumulative 30-day rainfall as a potential confounder in multivariable models but excluded it as it did not meet our criteria for inclusion in final adjusted models.

Finally, results from this study may not be generalizable outside of the study setting. The effectiveness (or lack thereof) of the intervention could be impacted by the specific assortment of pathogens circulating in the community or region, the WASH-specific

behaviors, practices and customs of the local population, the pre-existing or planned level of sanitation coverage, and the local population density (2).

While our results demonstrate that access to hygienic, privately shared onsite sanitation systems do not reduce enteric infection in young children in this setting, future analyses of soil-transmitted helminth infections, environmental enteric dysfunction (Chapter 4) and malnutrition will explore further potential health impacts of the intervention. Additionally, results from our analysis of children born into intervention sites, which demonstrated a substantial reduction in the risk of *Shigella* infection, suggest that any health impact of the intervention may require greater than two years post-implementation to be realized, or may require protection from birth to delay key infections.

The need for effective sanitation solutions may be most urgent in densely populated, low-income, unplanned communities like our study setting where almost ubiquitous fecal contamination drives extraordinarily high infection burdens. Unsurprisingly, pathogen transmission in these settings may be very complex and is likely driven by multiple interrelated pathways. While decades of sanitation and health research have demonstrated meaningful health gains following improvements in sanitation, the results of this study, and many of the recent large-scale, rigorous trials of sanitation interventions, suggest that that relationship between sanitation and health is complicated, difficult to measure, and may not be generalizable across diverse settings and populations. Further, in settings similar to our study sites, where sanitary conditions are poor and infection burden is high, more comprehensive WASH interventions that achieve higher coverage levels may be necessary to achieve rapid health gains.

CHAPTER 4

ASSESSMENT OF THE IMPACT OF AN ON-SITE SHARED SANITATION INTERVENTION ON MARKERS OF ENVIRONMENTAL ENTERIC DYSFUNCTION IN CHILDREN LIVING IN MAPUTO, MOZAMBIQUE

ABSTRACT

Background

The relationship between fecal contamination exposure and long-term health and well-being effects like stunting and cognitive deficiencies may be mediated, in part, by environmental enteric dysfunction (EED). EED is a subclinical disorder affecting the structure and function of the gut and believed to result from frequent and persistent enteric infection. We aimed to understand if access to new hygienic shared latrine infrastructure affected the concentration of biomarkers of intestinal inflammation and permeability in the stool of young children living in Maputo, Mozambique.

Methods & findings

The MapSan trial is an independent health impact evaluation of a privately shared on-site sanitation intervention (pour-flush latrines to septic tanks) in low-income, unplanned urban neighborhoods of Mozambique. We enrolled children <4 years old in intervention and control sites during the baseline (pre-intervention) phase and followed-up with them

12- and 24-months post-intervention. Enrollment was progressive and at each phase we enrolled all eligible children (children <5 years old at each post–baseline visit) and collected survey data and stool specimens for EED analysis. We measured the concentration of four biomarkers of EED in stool: alpha-1-antitrypsin, neopterin, myeloperoxidase, and calprotectin. Our main analyses included all enrolled children and sub-group analyses assessed intervention effects in children with longitudinal data (multiple data-points available including baseline) and children who were born into study sites post-intervention. We analyzed the 12- and 24-month phases separately and combined into a single follow-up phase. In our main analyses, the intervention increased the concentration of neopterin by 0.17 $\log_{10}$ nmol/L (95% CI: 0.07 - 0.27) by the 12-month follow-up and had a borderline effect on MPO concentration (0.09 $\log_{10}$ ng/mL, 95% CI: 0.00 - 0.18). We observed similar results in our longitudinal analysis of children available at both the baseline and 12-month phases. Concentrations of calprotectin (0.18 $\log_{10}$ ng/mL, 95% CI: 0.02-0.35) and neopterin (0.17 nmol/L, 95% CI: 0.01, 0.33) increased in children born into intervention sites by the 24-month follow-up visit. The intervention did not a have statistically meaningful effect on the concentration of alpha-1-antitrypsin, the only marker of intestinal permeability measured, but in contrast to the trend observed for markers of intestinal inflammation, the concentration of alpha-1-antitrypsin tended to decrease following intervention handover and use.

Conclusions

The intervention had an inconsistent effect on the concentration of EED biomarkers after 12- and 24-months of exposure. The etiology and pathophysiologic mechanisms

underlying EED have not been fully described, complicating its measurement. The lack of a formal case definition and of representative healthy reference concentrations for common EED biomarkers makes interpretation of effect estimates, especially interpretation of their potential clinical significance, complex. Our results highlight the urgency of filling these evidence gaps so we may begin designing and implementing sanitation interventions that prevent, or at least delay, the onset and severity of EED.

INTRODUCTION

Exposure to unsafe water and sanitation is a leading cause of diarrheal deaths in children less than five years old (16). While considerable progress has been made in reducing childhood death due to diarrhea since 2000 (16), measurement of diarrheal deaths, illness, or even disability-adjusted life years likely greatly underestimates the burden of disease attributable to poor water and sanitation conditions.

Repeated exposure to enteric pathogens via contact with fecal contamination is hypothesized to contribute to development of environmental enteric dysfunction (EED), a sub-clinical disorder affecting the structure and function of the small intestine (15,21,22,50,58,149,150). While children and adults with EED may not exhibit overt symptoms, EED has been associated with several downstream health effects such as linear growth (59,151,152), impaired cognitive development (153,154), poor oral vaccine

response (155,156), and increased susceptibility to future infection (20). There is currently no formal case definition for EED and histopathology of the intestinal wall remains the gold standard for informal diagnosis (49). Given the highly invasive nature of biopsying the gut, non-invasive biomarkers of several of the underlying pathophysiologic mechanisms or domains of EED have been proposed. These include markers of intestinal inflammation, permeability, damage and repair, microbial translocation, and systemic inflammation (22). However, not all domains have been linked to health outcomes, like stunting, and there is some discordance between the theoretical relationship between domains and the evidence in the literature. EED is an important target for WASH intervention evaluations given potential downstream health effects and its association with exposure to fecal contamination and enteric pathogens. However, unresolved questions about its case definition and measurement can complicate interpretation of results.

Few trials have measured the impact of WASH interventions on EED and no trials have specifically measured the impact of sanitation alone. Two small trials estimating the effects of a handwashing intervention and a community-based WASH intervention on found no effect (157,158). A recent large -scale cluster-randomized controlled trial in rural Bangladesh found modest effects of an intensive combined WASH intervention on markers of inflammation and permeability in very young children (< 3 months old) but limited effects in children aged 14 and 28 months (159). To date, no rigorous health impact evaluations have investigated the impact of sanitation on EED in urban areas.

As part of the MapSan trial, a controlled before-and-after health impact evaluation of an onsite, privately shared sanitation intervention in urban Mozambique (2), we aimed to evaluate the effect of the intervention, pour-flush latrines to septic tanks shared by 2 or more families, on four biomarkers of EED in young children.

METHODS

Study design and intervention

The MapSan Trial was an independent health impact evaluation of an onsite, privately shared sanitation intervention in low-income, densely populated, unplanned neighborhoods in Nlhamankulu district of Maputo, Mozambique. We used a controlled before-and-after (CBA) study design to measure the effect of a non-randomized, cluster-allocated sanitation intervention on child health. Prospective CBA studies like MapSan typically involve collecting data from participants in two study arms, an intervention group and a control group, at multiple time points before and after implementation of the intervention. This design enables us to estimate the effect of the intervention on outcomes while accounting for any secular trends in the study population over the study period which may affect outcome measures but be unrelated to the intervention (138). In MapSan, the intervention group was provided with pour-flush latrines to septic tanks and the control group continued using their original poor condition sanitation facilities. Data collection consisted of three phases – baseline (pre-intervention), 12-month follow-up,

and 24-month follow-up - each taking approximately one year to complete (Figures 2 & 3). During baseline, we completed enrollment and data collection activities in intervention and control sites in parallel to reduce any impact seasonality may have on our outcome measures. All sites were revisited approximately 12 and 24 months after baseline (Figure 3).

Water and Sanitation for the Urban Poor (WSUP) oversaw site selection and construction of intervention latrines independently of the MapSan study team. Due to the logistical and technical constraints of building latrine infrastructure in densely populated communities, intervention site selection was not random. WSUP performed site selection and implementation at the compound level. Compounds are groups of related or unrelated households which typically share outdoor living space and sanitation facilities. While compound-level sanitation facilities are shared by multiple households, they are not considered public latrines.

WSUP constructed one of two types of latrine infrastructure depending on compound population - communal sanitation blocks (CSBs) and shared latrines (SLs). CSBs serve larger compounds and include one toilet stall for each 20 beneficiaries living in the compound. CSBs also include other amenities such as a shared water connection, an elevated tank to store water from the intermittently piped water supply, a laundry facility, a rainwater harvesting system, and a well-drained area for bathing (Appendix A, Figure A2). Shared latrines serve fewer than 20 people and are single-stall infrastructure (Appendix A, Figure A3). All interventions, regardless of size, utilize the same type of sanitation technology: pour-flush latrines to septic tanks with soak-away pits (Appendix

A, Figure A1). Soak-away pits facilitate the infiltration of liquid waste into the ground. The septic tanks for both CSBs and SL infrastructure were sized to contain approximately two years of waste after which time emptying would be necessary.

Participants

Details of intervention and control site selection and participant eligibility and recruitment have been described previously (Chapter 3). Briefly, children aged 29 days to 48 months old were eligible for enrollment at baseline if they lived in a selected intervention or control compound and if their parents or guardians completed written informed consent. Intervention site selection was performed by WSUP which consulted with local government officials to identify potential sites for intervention. Final site selection was based on factors related to demographics, engineering constraints, and WASH conditions including use of poor sanitation, stated demand, number of beneficiaries, availability of a water piped water supply, location, space, and groundwater level. WSUP engineers completed site selection based on the above criteria and were charged with determining whether current sanitation conditions in prospective sites were considered "poor." Specifically, engineers assessed the type, functionality, and number of latrines. Latrines traditionally classified as "unimproved" (e.g. pit latrines without concrete slabs) were designated as poor sanitation, as were technologies typically considered "improved" if they had fallen into disrepair or where otherwise not functioning as intended (e.g. leaking or clogged pour-flush latrines, cracked slabs or slabs covered in dirt or debris). We identified control sites using a subset of WSUP's intervention selection criteria including sharing sanitation in poor condition, number of

beneficiaries, access to legal piped water supply, and hypothetical demand for improved sanitation. We attempted to identify control compounds with similar numbers of members as intervention compounds so that the population distribution of compounds in intervention and control compounds would be comparable. Control compounds must also have been home to at least one child less than 48 months old.

We completed enrollment and baseline data collection progressively between February 2015 and February 2016 as intervention latrines were constructed and scheduled for handover. We planned to visit intervention compounds within the two weeks prior to intervention handover. Due to unexpected delays in construction, we visited some compounds earlier than the scheduled two week window, but visited all compounds prior to latrine handover and use. We visited intervention and control compound at approximately the same rate during all three study phases to avoid the potential influence of seasonality on outcome measures. We scheduled intervention follow-up visits to be 12 months (±2 weeks) and 24 months (±2 weeks) from the date of intervention use. To preserve similarity in visit rates between intervention and control compounds, we scheduled a control follow-up visit within two weeks of each intervention follow-up visit. Children absent during the baseline visit were eligible for enrollment at the 12-month and 24-month visit if they resided in an intervention or control compound, if their parents or guardians consented to their participation, if they were between 29 days and 60 months old at the time of enrollment, and if they had moved into the compound more than six months prior to the visit or were born into the compound.

Participants and study enumerators were not blinded to intervention status during the study given the obvious visual components of the intervention. The WSUP implementation team and MapSan field enumerator team functioned independently and consisted of different individuals.

Procedures

Field data collection procedures have been previously described (Chapter 3). Field teams completed consent procedures, and completed survey data and specimen collection activities from eligible children at each phase. Prior to beginning data collection activities, enumerators secured verbal assent from the head of the compound or his or her spouse. We required the parents or guardians of eligible children to provide written, informed consent prior to initial enrollment of the child in the study. At each subsequent phase, field enumerators sought verbal assent for continuation in the study.

We used surveys to collect data on key socio-demographic factors, environmental conditions in the household and compound, and WASH practices and behaviors using questionnaires and spot-checks (Table 4). To measure relative household socio-economic status, we used an asset-based poverty calculator developed for and validated in Mozambique (75). Additional survey variables have been described previously (Chapter 2, Appendix B, Table B1).

We tried to collect stool specimens from each enrolled child at each phase regardless of reported symptomology. Enumerators provided each caregiver with stool collection supplies and returned the next day to collect the specimens. If a child did not have a

bowel movement prior to the schedule pickup, caregivers were asked to call the field team as soon as the child produced a stool specimen. We provided caregivers with pre-paid phone credit for this purpose or to request fresh collection supplies. Following collection, enumerators stored bulk stool specimens in coolers with cold packs and delivered them to the medical parasitology laboratory at the Mozambican Ministry of Health (MISAU/INS) within six hours. Laboratory technicians aliquoted the stools into up to four sterile tubes, depending on availability of material, and stored aliquots at -80°C until shipment. Approximately two times a year, archived stool samples were shipped on dry ice to the Georgia Institute of Technology in Atlanta, Georgia, USA where they were stored at -80°C until analysis. Each shipment included temperature probes to ensure continuity of the cold-chain during shipment.

We analyzed the concentration of four biomarkers of EED in bulk stool specimens using commercially available enzyme-linked immunosorbent assays (ELISAs): alpha-1-antitrypsin (AAT) (Biovendor, Karasek, Czech Republic), neopterin (NEO) (Genway, San Diego, CA, USA), myeloperoxidase (MPO) (ALPCO, Salem, NH, USA), and calprotectin (CAL) (ALPCO, Salem, NH, USA). AAT is a serum glycoprotein typically released during inflammatory responses (22,160). Because AAT is not synthesized in the gut, it is considered a marker of intestinal permeability and is a classic marker of protein-losing enteropathies (161). Further, AAT concentration has been associated with the presence of certain enteric pathogens of importance such as *Shigella*, *Campylobacter*, and *Salmonella* (58,162,163) and with poor linear growth outcomes (59). NEO, MPO and CAL are considered markers of intestinal (local) inflammation (22). NEO is mostly

produced by macrophages and dendritic cells in response to activation of a proinflammatory (Th1) immune response (22,59,164). NEO concentration has also been associated with growth deficits (59,60). MPO is an enzyme contained in neutrophils (a type of white blood cell) and its concentration is considered proportional to the concentration of neutrophils in a sample (165). MPO has been proposed as a marker of inflammatory bowel disease (IBD) (165), has been associated with the detection of some enteric pathogens including *Shigella*, enteroinvasive *E. coli*, *Campylobacter*, and adenovirus (58), and has been associated with linear growth deficits in children (58,59). Similar to MPO, CAL is a protein associated with neutrophils and its detection in stool may be indicative of migration of neutrophils to the intestine as part of an inflammatory immune response (22,166). Concentration of fecal CAL has been proposed as a marker of IBD, Crohn's disease, ulcerative colitis, and severity of diarrheal disease (166), however, its use may be limited by evidence that its concentration may be associated with breastfeeding status (22,66,167).

All ELISA kits used for biomarker measurement, with the exception of the NEO kit, are sandwich ELISAs and specifically tailored for use with stool. The NEO kit uses a competitive assay design and is marketed for use with urine, plasma or serum but has been used extensively to measure NEO concentrations in stool (58,59,113,159). We assayed all samples according to manufacturer instructions, except where noted. Each kit included standards and one high concentration and one low concentration control. We assayed all standards and controls in duplicate and used the results of the controls to validate the standard curve according to the manufacturers' instructions. We diluted

samples to a final dilution of 1:100 – 1:25,000 depending on the target marker. All samples we assayed for NEO were run in duplicate and the majority of samples we assayed for AAT, MPO, and CAL were run in duplicate. Due to budgetary constraints, between 12-16% of samples assayed for AAT, MPO, and CAL could not be tested in duplicate. Results from previous analyses indicated a small intra-assay coefficient of variation (4.6-6.6%) among sample replicates tested for AAT, MPO, and CAL. Samples assayed for NEO were always assayed in duplicate given the comparably higher intra-assay coefficient of variation of 15.6%. We re-assayed any sample that produced duplicate results more than 15% different than their mean. We re-diluted and re-assayed any sample with a concentration falling outside of the bounds of the standard curve or below the assay limit of detection defined by the manufacturer.

Due to an issue with diluent availability, we used 0.9% saline instead of the kit "assay buffer" as the sample diluent for approximately 15% of the samples assayed for NEO. Previous studies of EED using the same NEO ELISA kit used saline as a sample diluent (59,159,168). To correct for any potential discrepancy in results due to choice of diluent, we compared the results from 52 stools diluted in saline and assay buffer and assayed in parallel. We applied a minor adjustment to the results of samples diluted in saline in accordance with the results of the comparison study.

We were unable to run EED analyses for every stool specimen collected. We prioritized enteric pathogen analysis which meant that some samples did not have adequate material remaining for all four EED analyses. Similar to previous studies of fecal biomarkers of EED (22,58,59), we excluded liquid stools ("diaper samples") as there is some evidence

that diarrheal stools may dilute EED signals resulting in artificially low concentrations (169). We also could not assay fecal swabs for EED biomarkers as they were used for enteric pathogen analysis.

Outcomes

We pre-specified three of the four EED biomarkers as outcomes of the MapSan trial: AAT, MPO, and NEO (2). In addition to the pre-specified EED biomarkers, we also included CAL as a study outcome. We measured all four EED biomarkers in stool collected from children during the baseline, 12-month, and 24-month study phases. We combined the results of the AAT, MPO, and NEO assays into a composite EED score using the equation developed by Kosek et al (59). The EED score ranges from 0 – 10 with higher scores representing higher concentrations of the EED biomarkers.

Statistical analysis

Sample size calculations for the MapSan trial are described elsewhere (Chapter 3). While we attempted to analyze each bulk stool specimen by for all four EED measures, the sample size was ultimately limited by the availability of bulk stool material.

The main analysis included data collected from all children enrolled during the study regardless of phase of enrollment. However, if a compound received an intervention latrine after the end of the baseline phase (February 2016), only data collected prior to handover of the latrine was eligible for inclusion. For example, if a compound received an intervention latrine following the 12-month visit, any data collected during the 24-month visit is excluded from the present analysis. For the main analysis, we examine the

effect of the intervention at the 12-month and 24-month phases separately. As an exploratory analysis, we assess the effect of the intervention after combining data from the 12-month and 24-month visits into a single follow-up phase.

We also perform several sub-group analyses including an analysis of children with at least two phases of data (including baseline), and a comparison of children who were born into study sites post-intervention with children of a comparable age group at baseline. Our primary analyses and sub-group analyses use complete case data.

We used *t* tests and chi-square tests to examine differences in baseline demographic, socio-economic, and environmental and WASH characteristics between intervention and control sites. We used spearman rank correlation tests to assess pairwise correlations between concentrations of EED biomarkers. To evaluate the impact of the intervention on the concentrations of biomarkers of EED, we used a difference in difference (DID) approach. The DID approach allows us to estimate the effect of the intervention on EED while accounting for any secular trends in study population (via measurements of the control group) that may impact the outcome. The DID (or ratio of ratio) estimator is represented as the interaction of study arm and study phase dummy variables. By design, DID estimation accounts for baseline measures of the dependent or outcome variable – essentially serving as a form of built-in adjustment for baseline differences between the intervention and control arms (138). Prior to regression analysis, we log-transformed the concentrations of all four EED biomarkers. We used generalized estimating equations (GEE) with robust standard errors to fit linear regression models and account for compound level clustering effects. Given the hierarchical nature of the data, we clustered

on compound because it was both the unit of intervention allocation and the highest level of nested data (126). We based the main outcome assessment on multivariable models but also present the results from unadjusted models. While DID models account for baseline outcome measures, we refer to them as unadjusted to differentiate between models which include additional covariates (adjusted) and those which only include the DID estimator and its component variables (phase and study arm). We included covariates we determined *a priori* as potentially important, including child age, sex, breastfeeding status, caregiver's education, and household wealth score. Details on covariate selection have been presented previously (Chapter 3). We analyzed data from participants according to their exposure to the intervention latrine at each phase (as treated analysis). We assessed results of all analyses using traditional statistical hypothesis testing with a significance level of α=0.05. We did not adjust for multiple comparisons because the main analyses were pre-specified and sub-group analyses were exploratory in nature (127). We performed all statistical analyses with Stata version 14.1 (StataCorp, College Station, TX).

Ethics

The study protocol was approved by the Comité Nacional de Bioética para a Saúde (CNBS), Ministério da Saúde (333/CNBS/14), the Ethics Committee of the London School of Tropical Medicine and Hygiene (reference # 8345), and the Institutional Review Board of the Georgia Institute of Technology (protocol # H15160). The trial has been registered at ClinicalTrials.gov (NCT02362932).

RESULTS

Enrollment

During the baseline phase (February 2015 – February 2016), field enumerators enrolled 456 children and collected 362 stool samples in intervention compounds and 537 children and 395 stools in control compounds (Figure 2). Enumerators were able to follow up with 66.4% (303/456) intervention children and 59% (317/537) control children during the 12-month phase (March 2016 – April 2017) and enroll an additional 155 children in intervention compounds and 165 children in control compounds. We collected stools from 403 children in intervention compounds and 400 children in control compounds over the course of the 12-month phase. Over half (177/320) of new enrollees in intervention and control compounds had been born after the baseline visit, one third had moved into a study compound (102/320) and approximately 13% were enrolled during the 12-month visit for other reasons (absent or traveling during baseline, refused enrollment at baseline). During the 24-month phase, we collected data from 500 children in intervention compounds including 145 new enrollees, and from 505 children in control compounds of which a 159 were new enrollees. The 24-month phase lasted from April 2017 through May 2018 and we collected stool material from 459 intervention children and 464 control children. Of the children present at the 12-month visit, 133 and 167 were unavailable for data collection during the 24-month visit in intervention and control compounds, respectively. We collected baseline, 12-month, and 24-month data from 233/456 (51.1%) of intervention children originally enrolled at baseline and 217/537

(40.4%) of control children. Over 60% of control children (348/537) and 70% of intervention children (333/456) were available during at least one follow-up phase.

Baseline Population characteristics and balance

We have previously characterized the demographics, socioeconomic status, and environmental and WASH conditions and practices of the study population at baseline (Chapters 2 and 3). Briefly, child level demographics such as age, sex, breastfeeding status and level of caregiver education were balanced across study arms at baseline (Table 4). We observed some imbalance between arms in measures related to compound population such as number of households, population density of the immediate compound area (measured in persons/m^2), and number of water taps within compound boundaries. Some features of existing sanitation facilities varied among intervention and control compounds including the presence of drophole covers and sturdy latrine walls which were more common in intervention compounds than control at baseline. All households used a private municipal water tap as their primary drinking water source at baseline, though it was more common for the tap to be inside compound boundaries in intervention compounds. While intervention households scored slightly lower on our asset based wealth index than control compounds, the absolute difference was small.

The concentration of NEO and CAL, as well as the composite EED score were similar in both study arms at baseline (Table 10). The concentration of AAT was higher among intervention (-0.43 $\log_{10}$ mg/g) than control (-0.50 $\log_{10}$ mg/g) children at baseline. We observed the opposite trend for MPO: control children had higher concentrations of MPO (3.69 $\log_{10}$ ng/mL) than intervention children (3.62 $\log_{10}$ ng/mL). The concentrations of

all four biomarkers increased with age at baseline, both across arms (pooled intervention and control data) and in each arm independently.

Table 10: Mean EED score and log concentration of EED biomarkers at baseline and follow-up phases

	Baseline		12-month		24-month		Combined follow-up	
	Control	Intervention	Control	Intervention	Control	Intervention	Control	Intervention
log(AAT) (mg/g)	-0.50 (0.52) n=364	-0.43 (0.49) n=346	-0.57 (0.46) n=352	-0.57 (0.49) n=359	-0.61 (0.47) n=297	-0.66 (0.49) n=311	-0.59 (0.47) n=649	-0.61 (0.49) n=670
log(MPO) (ng/mL)	3.69 (0.43) n=370	3.62 (0.45) n=347	3.61 (0.50) n=352	3.60 (0.43) n=366	3.57 (0.56) n=307	3.49 (0.54) n=321	3.59 (0.53) n=659	3.55 (0.49) n=687
log(NEO) (nmol/L)	3.01 (0.55) n=349	2.95 (0.53) n=319	2.76 (0.52) n=312	2.84 (0.51) n=324	2.77 (0.50) n=250	2.73 (0.51) n=251	2.76 (0.51) n=562	2.79 (0.51) n=575
log(CAL) (ng/mL)	5.44 (0.49) n=368	5.42 (0.47) n=347	5.35 (0.58) n=348	5.34 (0.52) n=364	5.36 (0.57) n=298	5.37 (0.56) n=305	5.36 (0.58) n=646	5.35 (0.54) n=669
EED score	5.13 (2.56) n=343	4.93 (2.55) n=319	5.01 (2.56) n=310	5.03 (2.57) n=319	5.27 (2.58) n=237	4.79 (2.64) n=239	5.12 (2.57) n=547	4.92 (2.60) n=558

Data is presented as mean (SD), n=# of unique children and # of samples included in calculation. The variation in sample sizes for EED assays within a phase is due to exhaustion of sample material prior to completion of all 4 analyses.

We found positive pairwise correlations between all four of the EED biomarkers pooled across arms and in each arm independently at baseline, though the magnitude of correlation coefficient, ρ, varied among biomarker pairs (Table 11). Overall, NEO had the lowest pairwise correlations with all other biomarkers in pooled analysis, ranging from ρ=0.18 between NEO and AAT to ρ=0.39 between NEO and CAL. Myeloperoxidase and calprotectin had the highest correlation (ρ=0.76) of any two biomarkers. Results were similar when data from each arm was analyzed independently.

Table 11: Spearman rank correlation coefficients for pairwise comparisons of EED biomarkers at baseline.

	log(AAT) (mg/g)	log(MPO) (ng/mL)	log(NEO) (nmol/L)	log(CAL) (ng/mL)
log(AAT) (mg/g)	1.00			
log(MPO) (ng/mL)	0.35	1.00		
log(NEO) (nmol/L)	0.18	0.29	1.00	
log(CAL) (ng/mL)	0.41	0.76	0.39	1.00

Data is pooled across intervention and control arms. All pairwise correlations are significant at α=0.05 and have p-values<0.0001 for all pairs.

Intervention effect on all children (primary analysis)

Effect estimates for the main analysis and all sub-group analyses are the average expected change in biomarker concentration or EED score due to the intervention and are represented by the linear regression coefficient of the DID estimator. Effect point estimates and 95% confidence intervals falling below the null value (0) suggest a reduction in biomarker concentration or EED score and protective effect of the intervention. Confidence intervals which include the null value signify that the intervention had no statistically meaningful effect on the specified outcome, and estimates with confidence intervals above the null indicate an increase in biomarker concentrations (or EED score) due to the intervention.

At 12-months, we found no effect of the intervention on concentrations of AAT, CAL, or on the EED score. The intervention increased the concentration of NEO by 0.17 $\log_{10}$ nmol/L (95% CI: 0.07 - 0.27) in the 12-month adjusted analysis (Table 12). The intervention also had borderline weak effect on the concentration of MPO, which increased by 0.09 $\log_{10}$ ng/mL (95% CI: 0.00 - 0.18) in intervention children at the 12-month time-point.

The intervention had no effect on the concentration of AAT, MPO, CAL or the EED score in adjusted analyses at the 24-month follow-up phase. The concentration of NEO increased by 0.10 $\log_{10}$ nmol/L (95% CI: -0.01, 0.21) at 24-months, a smaller effect than observed in the 12-month analysis, but the confidence interval of the effect estimate included the null value (0). In unadjusted analyses, the intervention was associated with a

decrease in the concentration of AAT but the effect was weakened following adjustment (Table 12).

Results from the combined follow-up analysis were similar to results from the 12-month and 24-month individual analyses. The intervention resulted in an increase in the concentration of NEO by 0.14 $\log_{10}$ nmol/L (95% CI: 0.05-0.23) in adjusted analyses and a decrease in AAT concentration in unadjusted, but not adjusted, analysis (Table 12).

Table 12: Effect of intervention on log concentration of EED biomarkers and EED score in all enrolled children at 12-months, and 24-months and combined follow-up

	12-month		24-month		Combined follow-up	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
log(AAT) (mg/g)	-0.07 (-0.17, 0.03) n=1074 (1421)	-0.06 (-0.16, 0.04) n=1024 (1352)	-0.12 (-0.22,-0.02) n=1101 (1318)	-0.08 (-0.18, 0.03) n=997 (1188)	-0.09 (-0.18,-0.01) n= 1308 (2029)	-0.07 (-0.15, 0.02) n=1211 (1857)
log(MPO) (ng/mL)	0.07 (-0.03, 0.16) n=1079 (1435)	0.09 (0.00, 0.18) n=1028 (1366)	-0.01 (-0.11, 0.10) n=1111 (1345)	0.02 (-0.09, 0.13) n=1004 (1209)	0.03 (-0.05, 0.12) n= 1314 (2063)	0.06 (-0.02, 0.14) n=1215 (1886)
log(NEO) (nmol/L)	0.14 (0.02, 0.26) n=1003 (1304)	0.17 (0.07, 0.27) n=954 (1240)	0.03 (-0.10, 0.16) n=1007 (1169)	0.10 (-0.01, 0.21) n=909 (1051)	0.10 (-0.01, 0.20) n= 1225 (1805)	0.14 (0.05, 0.23) n=1129 (1649)
log(CAL) (ng/mL)	0.00 (-0.10, 0.11) n=1071 (1427)	-0.01 (-0.11, 0.09) n=1021 (1358)	0.03 (-0.08, 0.14) n=1097 (1318)	0.07 (-0.04, 0.17) n=991 (1185)	0.01 (-0.08, 0.10) n=1304 (2030)	0.02 (-0.06, 0.11) n=1204 (1855)
EED score	0.18 (-0.37, 0.72) n=998 (1291)	0.37 (-0.12, 0.86) n=950 (1229)	-0.31 (-0.90, 0.28) n=991 (1138)	0.03 (-0.54, 0.60) n=898 (1028)	-0.02 (-0.51, 0.46) n=1213 (1767)	0.23 (-0.21, 0.68) n=1122 (1620)

Data presented as regression coefficient estimates of DID estimator and 95% CI. n= # of unique children included in the analysis (# of unique samples analyzed).

Sub-group analysis: impact of intervention on EED biomarker concentration in children available at 2 (or more) phases

MapSan was originally designed as a prospective longitudinal cohort study but due to the larger than anticipated loss to follow-up, we were only able to follow a subset of all enrolled children through time. By analyzing that subset of children who were enrolled at baseline and available at one or more follow-up phase, we can simulate the analysis as originally planned. Prospective longitudinal cohort studies often have greater statistical efficiency than other study designs, such as repeated cross-sectional, assuming similar sample sizes (141).

The effect of the intervention on NEO concentration we observed in the main analysis of all children persisted in the 12-month sub-group analysis and resulted in an increase in NEO concentrations of 0.17 $\log_{10}$ nmol/L (95% CI: 0.03, 0.32) in adjusted analysis (Table 13). The intervention also had a marginal effect on MPO concentrations which increased by 0.12 $\log_{10}$ ng/mL (95% CI: -0.01, 0.26) in adjusted analyses. The intervention had no effect on concentrations of AAT or CAL or on the composite EED score at 12-months.

Sample sizes for the 24-month analysis were small due to loss to follow-up, and the intervention had no measurable effect on the concentration of the four EED biomarkers or the EED score (Table 13). By combining the results from the 12-month and 24-month phases, we assessed whether the intervention had any effect on children enrolled at baseline and available at one or more follow-up phases. The results were similar to those observed in the individual 12-month and 24-month analyses. The intervention resulted in

increased concentration of NEO by 0.16 $\log_{10}$ nmol/L (95% CI: 0.03, 0.30) in the adjusted analysis. The concentration of AAT was reduced in the adjusted combined analysis though the confidence interval just crossed the null (aRR -0.12, 95% CI: -0.25, 0.01). The effect observed on MPO in the 12-month analysis was attenuated in the combined analysis.

Overall, results from the sub-group analysis were consistent with results from the main analysis. The effect on NEO was consistently observed while other effects were less stable across analysis type and target.

Table 13: Effect of intervention on log concentration of EED biomarkers and EED score in children with stool samples available for analysis in two or more phases (including baseline).

	12-month		24-month		Combined follow-up	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
log(AAT) (mg/g)	-0.07 (-0.21, 0.07) n=347 (694)	-0.07 (-0.21, 0.07) n=328 (656)	-0.06 (-0.23, 0.11) n=217 (434)	-0.11 (-0.30, 0.07) n=191 (382)	-0.09 (-0.21, 0.03) n=409 (973)	-0.12 (-0.25, 0.01) n=360 (852)
log(MPO) (ng/mL)	0.1 (-0.03, 0.23) n=356 (712)	0.12 (-0.01, 0.26) n=338 (676)	0 (-0.18, 0.17) n=234 (468)	-0.02 (-0.21, 0.16) n=205 (410)	0.06 (-0.06, 0.17) n=421 (1011)	0.08 (-0.04, 0.21) n=370 (884)
log(NEO) (nmol/L)	0.15 (0.00, 0.29) n=301 (602)	0.17 (0.03, 0.32) n=286 (572)	0.09 (-0.11, 0.28) n=162 (324)	0.10 (-0.11, 0.30) n=142 (284)	0.13 (0.00, 0.26) n=349 (812)	0.16 (0.03, 0.30) n=313 (724)
log(CAL) (ng/mL)	0.02 (-0.11, 0.16) n=356 (712)	0 (-0.14, 0.14) n=337 (674)	0.01 (-0.17, 0.19) n=221 (442)	0.01 (-0.18, 0.2) n=194 (388)	0.01 (-0.11, 0.13) n=420 (997)	0.01 (-0.12, 0.13) n=369 (872)
EED score	0.42 (-0.29, 1.14) n=293 (586)	0.52 (-0.19, 1.24) n=279 (558)	-0.39 (-1.43, 0.66) n=147 (294)	-0.44 (-1.55, 0.67) n=130 (260)	0.08 (-0.56, 0.72) n=334 (774)	0.17 (-0.50, 0.83) n=303 (697)

Data presented as regression coefficient estimates of DID estimator and 95% CI. n= # of unique children included in the analysis (# of samples analyzed).

Sub-group analysis: impact of the intervention on EED biomarker concentrations in children born into the study

Analysis of children born into the study population post-intervention (or post-baseline visit in controls) provides estimates of the effect of the intervention on children who have spent their entire lives exposed to intervention. The 12-month sub-group analysis compared children less than one year old at the baseline visit with children less than one year at the 12-month follow-up visit who were reported as born into the study site. Similarly, the 24-month sub-group analysis included children less than two years at baseline and children less than two years at the 24-month visit who were recorded as born into a study site. The combined follow-up analysis combined observations from the 12- and 24-month analyses.

The intervention had no measurable effect on the concentration of any of the four EED biomarkers or the EED score in the 12-month analysis (Table 14). Effect estimates for the analyses were based on small sample sizes due to the narrow inclusion criteria. Stool material for EED analysis was only available from between 82 and 88 children (depending on EED analysis and split approximately evenly between study arms) who were born into the study and less than one year old by the 12-month visit. The majority of children included in the 12-month analyses were a part of the baseline comparison group (children <1 year old).

More children born into the study after baseline met the inclusion criteria for the 24-month phase analyses (138 – 164 depending on assay) but the majority of samples analyzed came from children in the baseline comparison group (children <2 years old at

baseline). Exposure to the intervention resulted in increased concentrations of NEO (0.17 $\log_{10}$ nmol/L, 95% CI: 0.01-0.33) and CAL (0.18 $\log_{10}$ ng/mL, 95% CI: 0.02-0.35) in adjusted analyses (Table 14). The intervention had no impact on AAT or MPO concentration or EED score. In the combined analysis, we observed a similar effect on NEO concentration but the effect on CAL was weakened (Table 14).

Table 14: Effect of intervention on log concentration of EED biomarkers and EED score in children born after baseline (compared to children of similar age at baseline) at 12-months, and 24-months and in combined follow-up data.

	12-month		24-month		Combined follow-up	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
log(AAT) (mg/g)	0.15 (-0.06, 0.37) n=265	0.15 (-0.06, 0.36) n= 260	-0.03 (-0.18, 0.12) n=556	0.02 (-0.13, 0.17) n=536	0.00 (-0.14, 0.13) n=602 (642)[1]	0.05 (-0.09, 0.18) n=586 (622)
log(MPO) (ng/mL)	0.00 (-0.19, 0.2) n=270	-0.01 (-0.20, 0.19) n= 265	0.02 (-0.15, 0.2) n=561	0.04 (-0.14, 0.22) n=540	0.02 (-0.12, 0.16) n=608 (649)	0.01 (-0.13, 0.16) n=591 (628)
log(NEO) (nmol/L)	0.16 (0.00, 0.31) n= 261	0.14 (-0.02, 0.30) n= 256	0.08 (-0.10, 0.26) n=521	0.17 (0.01, 0.33) n=502	0.12 (-0.02, 0.26) n=571 (606)	0.16 (0.04, 0.29) n=556 (587)
log(CAL) (ng/mL)	0.12 (-0.11, 0.34) n=263	0.08 (-0.13, 0.30) n= 258	0.15 (-0.01, 0.32) n=553	0.18 (0.02, 0.35) n=534	0.12 (-0.02, 0.25) n=598 (635)	0.14 (-0.01, 0.28) n=582 (616)
EED score	0.91 (-0.24, 2.06) n= 256	0.89 (-0.25, 2.03) n= 251	0.41 (-0.49, 1.31) n=513	0.62 (-0.23, 1.47) n=495	0.49 (-0.28, 1.25) n=563 (596)	0.60 (-0.14, 1.35) n=549 (578)

Effect estimates presented as coefficient of DID estimator (95% CI), n represents # of unique children in analysis unless otherwise specified. [1] n=# of unique children (# of samples analyzed). Children born into the study after baseline but before 12-month were eligible for inclusion in the 24-month follow-up analysis and provided data from two stools samples (12-month and 24-month) in the combined analysis.

DISCUSSION

The intervention had an inconsistent effect on the concentrations of the EED biomarkers after 12 and 24 months of exposure. Unexpectedly, the intervention appeared to increase the concentrations of some markers of intestinal inflammation in a subset of the analyses. The concentration of NEO, a marker of cellular immune system activation released during pro-inflammatory responses, increased in the 12-, 24-month and combined follow-up analyses, though the effect in the 24-month analysis was smaller and only borderline significant. NEO results from the two sub-group analyses were largely consistent with results from the main analysis. The intervention also increased the concentration of CAL, another marker of intestinal inflammation, among children born into the study before the 24-month visit, though we did not observe an effect on CAL in any other phase or analysis. All other potential effects were either borderline significant or only observed in unadjusted analyses, including the observed reductions of AAT, a marker of intestinal permeability.

The concentrations of all four biomarkers decreased in both intervention and control children between baseline and the 12-month follow-up. Concentrations of AAT and MPO continued to decrease in both arms after the 12-month visit but the concentrations of NEO and CAL leveled off in the 24-month measures. These decreases are likely due to the aging of the participants over the course of the study given age effect we observed in our baseline data. Even with these decreases, the concentration of all markers, with the exception of AAT, remained higher than concentrations reported for healthy controls

(59,170,171), similar to findings of other studies of EED in children in LMICs (59,113,159,172). Concentrations of AAT exceeded the healthy reference level (>0.27 mg/g) in both intervention and control at baseline, decreased to the healthy reference standard by 12-month, and fell below it by the 24-month visits (59,170). The healthy reference concentrations used for comparison here, and in many other studies of EED in similar settings, are from of adults or children living in high-income countries. These reference values may not be representative of what a "healthy" gut looks like in all age groups or settings. Future research should focus on establishing healthy reference concentrations for these and other biomarkers of EED in a variety of settings and populations.

The onset of EED is hypothesized to be caused by repeated exposure to enteric pathogens in fecally contaminated environments. Several observational studies have noted associations between increased fecal biomarker concentrations and poor sanitary conditions (113,159,168), but to date, few large-scale sanitation intervention trials have published results on EED. The WASH-Benefits Bangladesh trial, which measured the impacts of nutrition, WASH, and combined WASH+nutrition interventions on markers of EED, demonstrated reductions in NEO and lactulose and mannitol (markers of intestinal permeability) in children three months of age in all three intervention arms, but those reductions were not sustained in the WASH-only arm when the children were remeasured at 14 months and 28 months old (159). The NEO effect estimates for the nutrition-only and WASH+nutrition arms were similar at age 14 months, suggesting nutrition, and not WASH, was driving the reductions. Further, the concentrations of MPO and AAT

increased in the WASH-only arm in children aged 28 months. The authors hypothesize that the interventions may have delayed the onset of EED in young children but could not prevent it. Therefore, "peak deterioration" of the gut may have occurred later in intervention children, resulting in the observed increased concentrations of MPO and AAT at 28 months and the attenuation of effect on NEO, lactulose, and mannitol observed at younger ages. This hypothesis could help explain the increased concentrations of NEO and CAL we observed in our sub-group analysis of children born into the intervention by the 24-month visit. Unfortunately, we are not able to assess the plausibility of this theory because we do not have the statistical power to test whether the intervention had an effect on very young children (<3 months or <6 months) born into the study.

Certain enteric pathogen infections have been associated with increases and decreases in the concentration of different EED biomarkers (58). For example, infection with *Campylobacter*, *Giardia*, and *Y. pestis* have all been associated with decreased concentrations of NEO (58). The onset and severity of EED may be driven by exposure to fecal contamination generally but also by exposure to and infection with specific pathogens. While our cross-sectional measurements of infection cannot provide a full accounting of all infections which may have contributed to EED over the course of the study, we can examine the potential associations of pathogens measured concurrently with EED biomarkers. For example, in contrast with previous findings, we observed that *Campylobacter* infection was associated with increases in NEO concentration in pooled analysis. Further, stratification of NEO effect analyses by *Campylobacter* detection status

results in changes in both the magnitude of the effect of the intervention on NEO and the strength of the association. Past or present infection with pathogens associated with specific EED biomarkers may modify the effect of the intervention on EED.

Strengths and limitations

In addition to the strengths and limitations described previously for the MapSan trial in general (Chapter 3), there are several specific to this analysis. First, as there is no formal case definition for EED (49), we cannot draw conclusions as to whether an individual child has EED or not. The lack of a case definition for EED, coupled with the continuing examination of its etiology, complicates the interpretation of EED results from health impact trials such as MapSan which often seek to understand clinical significance of effect estimates or how they translate to changes in exposure. Formalizing a case definition for EED has been difficult, in part, because so many candidate markers have been proposed and comparison to the current "gold standard" method for assessing EED, examining gut histopathology, is difficult given the highly invasive nature of collecting specimens. We chose to examine the previously validated fecal markers of EED because their widespread use would facilitate comparison across studies. Also, compared with other specimen types used in EED analyses, like blood and urine, collection of stool is less invasive and logistically simpler to collect. However, the biomarkers we measured, which represent a fraction of candidate markers, may not provide a comprehensive accounting of a child's EED status as they only measure two of the five domains of EED (Table 1) (22).

Second, breastfeeding status likely plays an important role in the development and severity of EED. Breast milk can aid in gut maturation (173), impede pathogen adhesion, diminish intestinal inflammation (174), and increase concentrations of CAL (175). The practice of breastfeeding can modulate a child's exposures to fecal contamination. For these reasons, we measured breastfeeding status and included it as an important covariate in our models. However, capturing information about breastfeeding status in a cross-sectional survey may not provide enough resolution or detail and we cannot dismiss the potential for residual confounding by this important variable.

Third, we had to dilute a subset of samples prepared for the NEO assay with saline in place of the diluent provided with the ELISA kit. Saline has been used as the diluent for NEO assays in numerous studies (59,159,168). To verify that the short-term change in diluent would not affect our results, we assayed samples diluted in both saline and kit diluent in parallel, compared the results, and applied a small correction factor based on our comparison to our saline results. We ran a similar proportion of stools from intervention and control children with the saline diluent at each phase and the use of saline should not differentially affect the results.

Finally, because we excluded diarrheal stools and fecal swabs from our EED analyses and prioritized use of bulk stool material for enteric pathogen analysis, our sample sizes for EED analyses were lower than for pathogen analysis. This reduced sample size, coupled with the noisy nature of the EED measurements, may have limited our ability to detect small changes in EED concentrations, especially in sub-group analyses.

We found no evidence that the intervention improved indicators of EED in young children in this setting. EED's complex etiology and the numerous interrelated pathophysiologic mechanisms (and their representative biomarkers) used to describe it, complicate its measurement (22) and our understanding of how to prevent, or at least delay, its onset and severity. Our findings emphasize the necessity of filling these evidence gaps so that we may design interventions or treatments to prevent, reverse, or delay EED.

APPENDIX A

INTERVENTION DESIGN

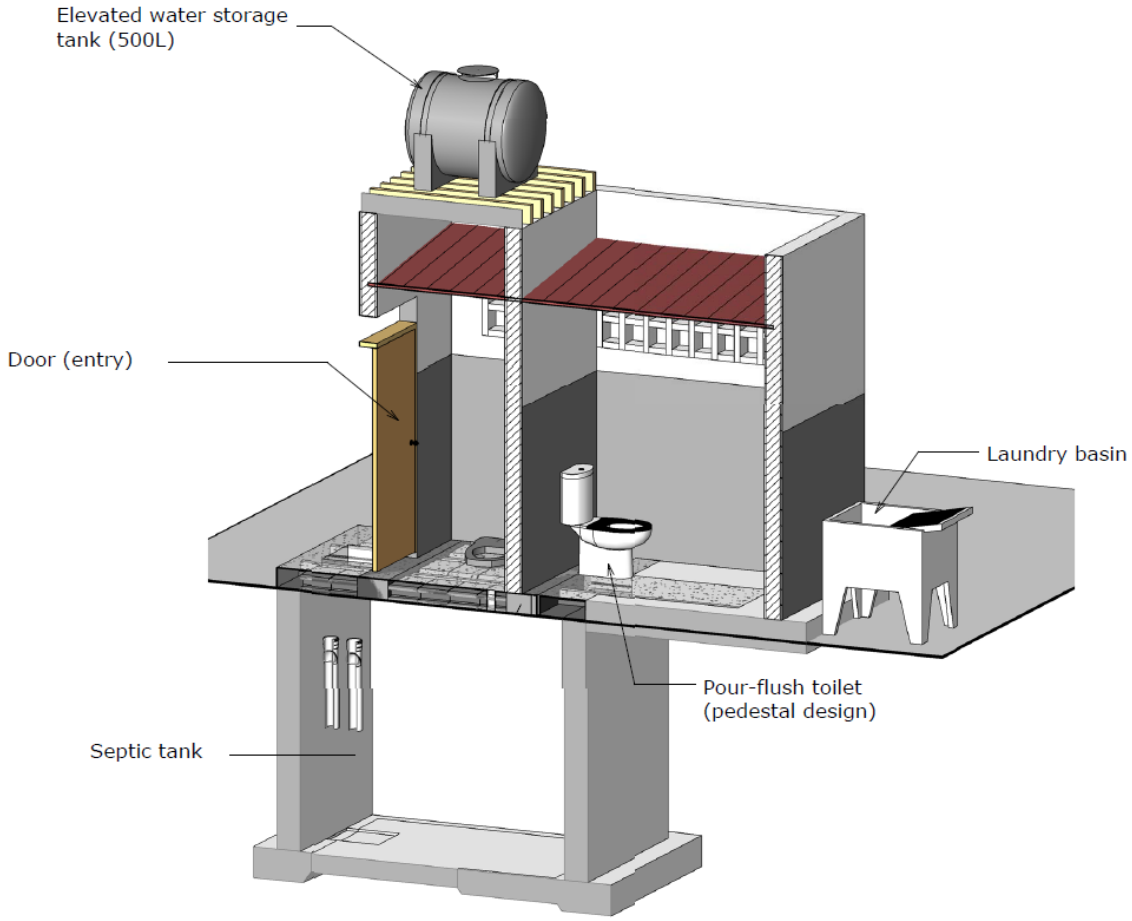

Figure A1: Original schematic of communal sanitation block design from WSUP. Pictured: 2 latrine stalls, 2 pour-flush toilets, septic tank, elevated water storage tank, laundry basin, door. Not pictured: soakaway pit.

Figure A2: Photo of communal sanitation block as constructed

Figure A3: Photo of shared latrine as constructed

APPENDIX B

CHAPTER 2 SUPPLEMENTARY INFORMATION

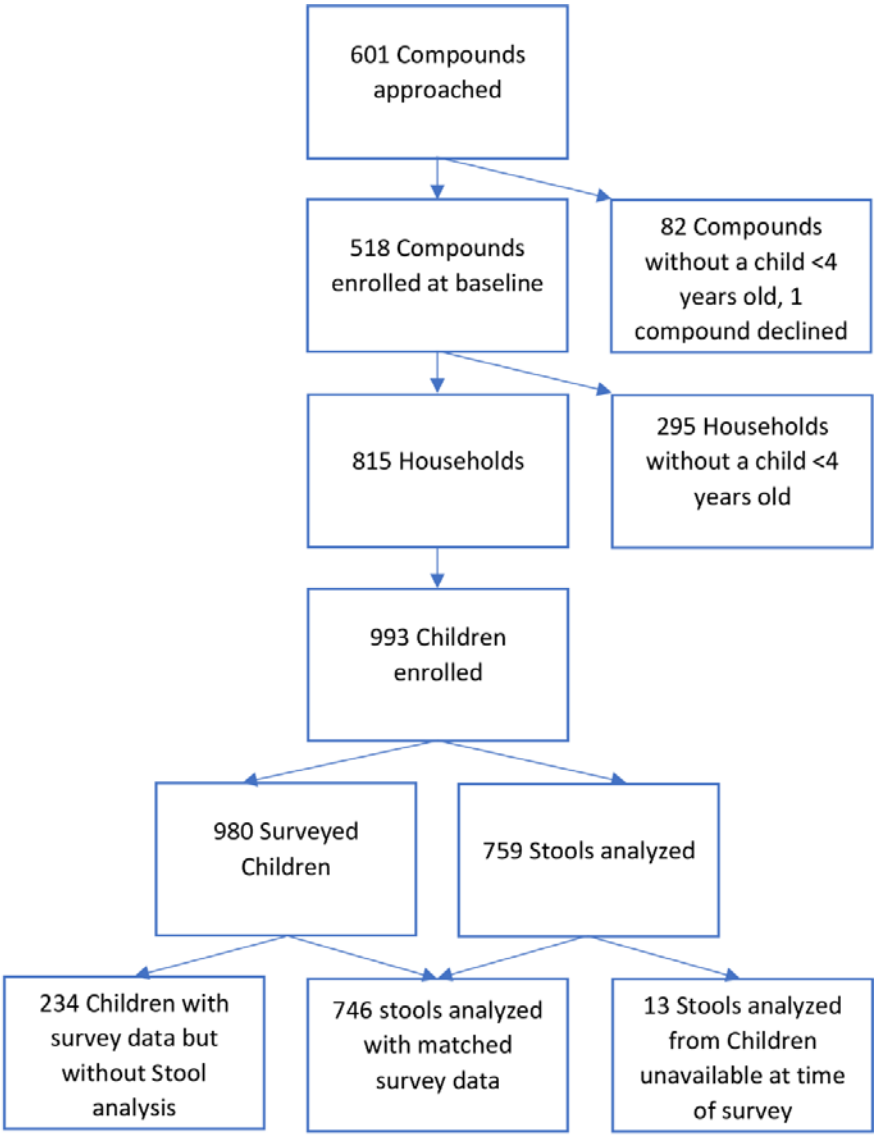

Figure B1: MapSan baseline enrollment profile

Table B1. Definitions and coding schemes for analysis variables.

Variable Name	***% Missing***	***Variable definition and format***
Latrine superstructure	1.9	Binary variable that is 1 when there was a wall around the latrine that provided privacy and security, 0 otherwise
Drophole cover present*	1.9	Binary variable that is 1 if the latrine drophole was covered, 0 otherwise.
Ventpipe present	1.8	Binary variable that is 1 if the latrine pit was vented, 0 otherwise.
Pedestal or slab present	2.2	Binary variable that is 1 if the latrine had a slab or a concrete/masonry pedestal.
Composite sanitation score	4.0	Ordinal variable ranging from 0-4. One point each awarded for presence of latrine superstructure, drophole cover, ventpipe, or slab/pedestal.
HHs sharing latrine	4.3	An ordinal variable that was 0 if two or fewer households shared a latrine, 1 if three to five households shared a latrine, and 2 if more than five households shared a latrine.
Disposal of child feces in latrine*	1.3	Binary variable that is 1 if children's feces were disposed of in a latrine, 0 otherwise (soiled diapers thrown on the trash heap).
Standing water in compound	1.9	Binary variable that is 1 if the field team observed standing water in the compound at the time of survey, 0 otherwise.
Wastewater in compound	1.9	Binary variable that is 1 if the field team observed waste water in the compound at the time of survey, 0 otherwise.

Table B1 (continued).

Visible feces or used diapers	*1.9*	*Binary variable that is 1 if the field team observed human feces or soiled diapers in the compound at the time of survey, 0 otherwise.*
Compound floods when it rains	1.9	Binary variable that is 1 if the head of compound reported that the compound had a tendency to flood due to rain, 0 otherwise.
Compound hygiene score	1.9	Ordinal variable ranging from 0-3. One point each awarded for presence of standing or leaking wastewater in compound, presence of visible feces or soiled diapers on compound grounds, and reported flooding.
Drinking water tap on compound grounds	1.8	Binary variable that is 1 if there was a water tap within the compound, 0 otherwise.
Any animal in compound	0.0	Binary variable that is 1 if the head of compound reported that 1 or more animal lived in the compound, 0 otherwise.
Dogs in compound	0.0	Binary variable that is 1 if the head of compound reported that 1 or more dogs lived in the compound, 0 otherwise.

Supplemental Information B1

We used two MI models for this analysis; the first any infection as the dependent variable and the second included the outcome measures of any bacterial infection, any protozoan infection, and any viral infection. All risk factors and covariates used in the analysis model were included in the MI model to ensure consistency and limit introduction of bias during imputation. Other than the difference in dependent variable, all other components of the MI models were similar. We used MI with chained equations (MICE) to create 50 complete datasets (m=50). We used MICE given its flexibility in handling binary and categorical variables. Logit distributions were used for all binary variables, ordered logistic for categorical, and predictive mean matching (knn=10) for any continuous or semi-continuous variables. We used an inclusive model with 6 auxiliary variables in the MI models given their observed or presumed association with values or missingness of any of the model variables.

Table B2: Crude and adjusted risk ratios and 95% confidence intervals for associations of caregiver reported diarrhea and enteric infection. Multivariable models are adjusted for child age and sex, caregiver education, household wealth, and breastfeeding practices.

	Crude risk ratio, n=652	Adjusted risk ratio, n=635
Any Infection (≥1 infections)	1.11 (0.65 – 1.89)	1.32 (0.75 – 2.31)
Any Viral Infection	1.37 (0.82 – 2.29)	1.34 (0.79 – 2.27)
Any Bacterial Infection	1.19 (0.75 – 1.89)	1.35 (0.85 – 2.14)
Any Protozoan Infection	0.92 (0.63 – 1.34)	1.07 (0.70 – 1.61)
Bacteria		
Shigella	0.95 (0.65 – 1.37)	1.20 (0.78 – 1.86)
ETEC LT/ST	0.88 (0.58 – 1.34)	0.88 (0.57 – 1.35)
Salmonella	0.96 (0.62 – 1.49)	0.90 (0.57 - 1.44)
Campylobacter	1.07 (0.58 – 1.98)	0.98 (0.52 – 1.86)
Clostridium difficile, Toxin A/B	1.25 (0.59 – 2.65)	1.11 (0.54 – 2.31)
Escherichia coli O157	1.13 (0.47 – 2.75)	1.27 (0.51 – 3.15)
STEC stx1/stx2	0.54 (0.08 – 3.75)	0.58 (0.08 – 3.97)
Yersinia enterocolitica†	-	-
Vibrio cholerae†	-	-
Protozoa		
Giardia	0.95 (0.65 – 1.38)	1.11 (0.72 – 1.71)
Cryptosporidium	1.56 (0.72 – 3.36)	1.46 (0.68 – 3.15)
Entamoeba histolytica†	-	-
Virus		
Norovirus GI/GII	1.74 (1.02 – 2.97)*	1.76 (1.03 – 3.02)*
Adenovirus 40/41	0.70 (0.20 – 2.41)	0.40 (0.07 – 2.18)
Rotavirus A	0.79 (0.15 – 4.14)	0.78 (0.15 – 3.96)

†Prevalence <0.01. Model not performed. *p<0.05

Table B3. Crude and adjusted risk ratios from the multiple imputation risk factor analyses for four measures of enteric infection: any enteric infection, any bacterial infection, any protozoan infection, and any viral infection. Multivariable models are adjusted for child age and sex, caregiver education, household wealth, and breastfeeding practices.

	Any Infection		Any Bacterial Infection		Any Protozoan Infection		Any Viral Infection	
n=993	RR	aRR	RR	aRR	RR	aRR	RR	aRR
Latrine superstructure	0.93 (0.86-1.00)*	0.94 (0.88-1.01)	0.94 (0.85-1.04)	0.95 (0.86-1.04)	0.82 (0.69-0.97)*	0.87 (0.75-1.01)	0.79 (0.52-1.20)	0.77 (0.51-1.18)
Drophole cover present	0.94 (0.89-1.00)	0.96 (0.91-1.02)	0.90 (0.83-0.98)*	0.92 (0.85-0.99)*	0.94 (0.83-1.08)	0.99 (0.88-1.12)	0.97 (0.68-1.39)	0.93 (0.65-1.34)
Ventpipe present	0.99 (0.91-1.08)	1.00 (0.92-1.09)	0.98 (0.86-1.11)	0.98 (0.87-1.11)	0.95 (0.77-1.18)	0.95 (0.79-1.15)	1.07 (0.64-1.78)	1.09 (0.67-1.80)
Pedestal or slab present	0.97 (0.91-1.03)	0.97 (0.92-1.03)	1.00 (0.92-1.10)	1.00 (0.92-1.10)	0.96 (0.83-1.12)	0.97 (0.84-1.11)	0.99 (0.67-1.45)	0.96 (0.66-1.40)
Latrine improvement index	0.97 (0.95-1.00)*	0.98 (0.96-1.01)	0.97 (0.93-1.00)	0.98 (0.94-1.01)	0.95 (0.89-1.00)	0.97 (0.92-1.02)	0.97 (0.84-1.12)	0.97 (0.84-1.12)
HHs sharing latrine								
HH<=2	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
3-5 HH	0.95 (0.89-1.03)	0.97 (0.91-1.04)	0.95 (0.86-1.06)	0.98 (0.88-1.08)	0.96 (0.80-1.16)	1.02 (0.87-1.20)	1.00 (0.62-1.61)	0.99 (0.61-1.61)
> 5 HH	0.94 (0.86-1.03)	0.98 (0.90-1.06)	0.90 (0.79-1.03)	0.95 (0.83-1.07)	0.98 (0.79-1.22)	1.11 (0.92-1.35)	0.94 (0.51-1.72)	0.93 (0.50-1.72)
Disposal of child feces in latrine	1.15 (1.09-1.22)*	1.00 (0.95-1.06)	1.17 (1.08-1.27)*	1.02 (0.93-1.12)	1.73 (1.53-1.97)*	1.07 (0.94-1.22)	0.79 (0.52-1.20)	0.95 (0.58-1.57)
Standing water in compound	0.98 (0.87-1.11)	0.96 (0.86-1.08)	0.94 (0.77-1.13)	0.92 (0.77-1.11)	1.13 (0.93-1.38)	1.04 (0.87-1.25)	0.65 (0.29-1.45)	0.69 (0.31-1.51)
Wastewater in compound	1.06 (0.99-1.13)	1.05 (0.99-1.12)	1.07 (0.98-1.16)	1.07 (0.98-1.16)	1.10 (0.95-1.28)	1.09 (0.96-1.25)	1.05 (0.72-1.53)	1.05 (0.72-1.52)

Table B3 (continued).

Visible feces or used diapers	1.08 (1.02-1.14)*	1.07 (1.01-1.13)*	1.07 (0.98-1.16)	1.06 (0.98-1.15)	1.12 (0.98-1.28)	1.08 (0.96-1.22)	0.84 (0.58-1.23)	0.88 (0.61-1.28)
Compound floods when it rains	1.00 (0.94-1.06)	1.00 (0.94-1.06)	0.98 (0.90-1.07)	0.98 (0.91-1.07)	0.95 (0.82-1.10)	0.94 (0.83-1.07)	1.19 (0.81-1.74)	1.20 (0.82-1.76)
Compound sanitary score	1.03 (1.00-1.06)	1.02 (1.00-1.05)	1.02 (0.98-1.07)	1.02 (0.98-1.06)	1.04 (0.97-1.11)	1.03 (0.97-1.09)	1.01 (0.85-1.19)	1.01 (0.85-1.19)
Drinking water tap on compound grounds	0.98 (0.91-1.04)	0.97 (0.91-1.03)	0.97 (0.89-1.06)	0.97 (0.88-1.06)	0.90 (0.77-1.06)	0.86 (0.75-0.99)*	0.77 (0.51-1.14)	0.78 (0.53-1.16)
Any animal in compound	1.03 (0.96-1.09)	1.01 (0.96-1.08)	1.03 (0.95-1.13)	1.02 (0.93-1.11)	0.99 (0.87-1.14)	0.95 (0.84-1.08)	1.42 (0.92-2.21)	1.43 (0.92-2.21)
Dogs in compound	0.96 (0.86-1.08)	0.96 (0.86-1.07)	1.08 (0.96-1.22)	1.07 (0.95-1.21)	0.83 (0.60-1.15)	0.80 (0.61-1.06)	1.27 (0.66-2.42)	1.19 (0.64-2.22)
Chickens or ducks in compound	1.01 (0.93-1.10)	0.99 (0.91-1.08)	1.01 (0.90-1.13)	0.99 (0.89-1.11)	1.04 (0.86-1.24)	0.98 (0.83-1.15)	0.96 (0.55-1.68)	0.98 (0.56-1.71)
Cats in compound	1.02 (0.96-1.09)	1.02 (0.96-1.08)	1.03 (0.95-1.12)	1.02 (0.94-1.10)	0.98 (0.86-1.12)	0.95 (0.84-1.07)	1.35 (0.90-2.04)	1.36 (0.91-2.04)
HH floor is covered	0.94 (0.85-1.04)	0.98 (0.88-1.08)	0.97 (0.83-1.14)	1.03 (0.87-1.21)	0.87 (0.68-1.12)	0.88 (0.71-1.09)	0.54 (0.32-0.91)*	0.56 (0.30-1.04)
Household crowding, > 3 persons/room	1.00 (0.93-1.08)	0.97 (0.90-1.05)	1.04 (0.94-1.15)	1.01 (0.90-1.12)	0.86 (0.71-1.04)	0.83 (0.69-1.00)	1.56 (1.02-2.40)*	1.54 (0.96-2.45)
Compound specific population density								
1 (least dense)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
2	1.07 (0.96-1.19)	1.05 (0.95-1.16)	1.05 (0.91-1.20)	1.03 (0.90-1.17)	1.05 (0.86-1.29)	1.02 (0.84-1.22)	1.25 (0.70-2.22)	1.23 (0.69-2.19)
3	1.06 (0.96-1.17)	1.05 (0.95-1.15)	1.11 (0.97-1.26)	1.10 (0.97-1.24)	1.00 (0.80-1.25)	0.98 (0.81-1.20)	1.03 (0.56-1.91)	1.04 (0.56-1.90)

Table B3 (continued).

4	1.07 (0.97-1.18)	1.05 (0.95-1.16)	1.04 (0.90-1.20)	1.02 (0.89-1.18)	1.03 (0.83-1.29)	1.00 (0.82-1.22)	1.30 (0.69-2.43)	1.26 (0.67-2.38)
5 (most dense)	1.10 (1.00-1.21)	1.09 (1.00-1.20)	1.06 (0.92-1.22)	1.05 (0.91-1.21)	0.98 (0.78-1.23)	1.02 (0.83-1.25)	1.43 (0.79-2.57)	1.32 (0.72-2.41)
Cumulative rainfall last 30 days, terciles								
1 (least rain)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
2	0.98 (0.92-1.05)	0.98 (0.92-1.05)	0.95 (0.86-1.04)	0.95 (0.86-1.04)	1.00 (0.85-1.18)	0.99 (0.86-1.14)	1.05 (0.66-1.65)	1.06 (0.67-1.68)
3 (most rain)	0.94 (0.88-1.02)	0.95 (0.88-1.02)	0.96 (0.86-1.06)	0.96 (0.87-1.06)	1.04 (0.88-1.21)	1.04 (0.90-1.20)	1.17 (0.74-1.87)	1.18 (0.74-1.88)
Child age								
1-11 months	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
12-23 months	1.21 (1.10-1.33)*	1.12 (1.00-1.26)*	1.13 (1.00-1.28)	1.05 (0.90-1.21)	2.74 (1.99-3.78)*	2.14 (1.49-3.09)*	0.85 (0.55-1.30)	0.75 (0.43-1.30)
24-48 months	1.34 (1.22-1.47)*	1.21 (1.07-1.37)*	1.28 (1.14-1.43)*	1.15 (0.98-1.35)	4.07 (2.99-5.53)*	2.89 (1.97-4.24)*	0.65 (0.42-0.98)*	0.55 (0.30-1.03)
Child gender, female	1.04 (0.98-1.10)	1.04 (0.98-1.10)	1.07 (0.99-1.15)	1.07 (0.99-1.15)	0.99 (0.87-1.12)	0.99 (0.88-1.11)	1.55 (1.09-2.20)*	1.53 (1.08-2.17)*
Any breastfeeding	0.79 (0.73-0.85)*	0.87 (0.79-0.96)*	0.81 (0.73-0.89)*	0.92 (0.81-1.04)	0.34 (0.27-0.43)*	0.49 (0.38-0.64)*	1.18 (0.83-1.70)	0.89 (0.54-1.46)
Caregiver completed primary school	0.95 (0.89-1.00)	0.98 (0.93-1.04)	0.99 (0.92-1.08)	1.03 (0.95-1.12)	0.84 (0.73-0.96)*	0.91 (0.79-1.04)	1.10 (0.77-1.56)	1.10 (0.78-1.57)

APPENDIX C

SURVEY TOOLS

Table C1: Baseline Compound Questionnaire. This questionnaire has been uploaded onto Magpi website and all data is collected electronically through the Magpi android mobile application.

IDs:*WSUPID*	WSUP Compound ID (given by WSUP spreadsheet)	
bairid	Bairros ID (official)	|__|__|
quartid	Quarteiro number (official)	|__|__|
clusterid	Intervention or control compound (2) Intervention (3) Control	|__|
Intervention latrinetype	If intervention compound, what type of latrine will the compound receive? Communal Sanitation Block Shared Latrine	
compid	Compound number (given by study). 4 digits long. Intervention start with "2", control start with "3"	|__|__|__|__|
fwid	Field worker ID	|__|__|
compsize	**ASK:** how many residents, including children, are living in this compound right now? If>=12 – **CONTINUE QUESTIONNAIRE** If < 12 – **STOP HERE**	|__|__|__|
compstrat	**Tick size category**	11-20 |__| 21-60 |__| 61+ |__|
anychild	**ASK: is there any child under 4 years (48 month) living in this compound right now?** (1)Yes (0) No – **STOP HERE**	|__|
compenr	**Compound is enrolled** (1)Yes (0) No – **STOP HERE**	|__|
complat	**OBSERVE/ASK Number of latrines/drop holes**	|__|__|
watpoint	**OBSERVE/ASK Number of water points within compound**	|__|__|
latwall	**OBSERVE: Do the latrines have stone walls?** (1)Yes (0) No	|__|

electr	**OBSERVE/ASK: is there electricity in the compound that normally functions?** (1)Yes (0) No	|__|
watstand	**OBSERVE/ASK: is there standing water in the compound** (1)Yes (0) No	|__|
wastewat	**OBSERVE: is there open waste water near the latrines or leaking from the latrines** (1)Yes (0) No	|__|
animal	**OBSERVE/ASK: what animals are in the compound Select all that are present** **(0) None** **(1) Dog** **(2) Cat** **(3) Chicken/duck** **(4) other**	|__|
faeces	**OBSERVE: Do you see faeces or used diapers on the ground or on a rubbish heap?** (1)Yes (0) No	|__|
flooding	**ASK: does this compound get flooded in the rainy season?** (1)Yes (0) No	|__|

Table C2: Baseline household and child questionnaire

childu5	**ASK:** is there a under 4 years (48 months) living in this household right now? (1) yes – **CONTINUE QUESTIONNAIRE** (0) no – **STOP HERE**	|__|
read	**ASK:** Have you read him/her the consent form? (1) yes – **CONTINUE QUESTIONNAIRE** (0) no-one is available to read it to – **STOP HERE**	|__|
cons	**ASK:** Does the respondent agree? (1)Yes (0) No – **STOP HERE**	|__|
resp	**OBSERVE/ASK**: Who is the respondent? Note: If possible, the mother or female caregiver should be the respondent. (1) Female head of household (2) Male head of household (3) female caregiver (4) male caregiver (5) other respresentative	|__|
hhcontact	**Name of respondent**	---------- ---------- ---------
hhhead	**Name of HH head**	---------- ---------- ---------
hhtel	**Phone number of HH contact** **Enter 0 for no phone.**	|__|__|__|__|__|__|__|__|__|__|__|__|__|

headedu	**ASK:** what education did the HH head receive?	None Some primary Primary completed Some secondary Secondary completed Some technical training Technical training completed Some higher education Higher education completed Refused question Don't know	0 1 2 3 4 5 6 7 8 88 99

hhsize	**ASK:** how many people sleep in your household?	|__|__|
Hhroom	**ASK:** how many bedrooms are in the house?	
Hhbeds	**ASK:** how many beds does this household have (single, double, bunkbeds, or for children)? (1) One (2) Two or more (0) none	
car	**ASK:** does this HH own a working bicycle, motocycle, or car? (1) yes, bicycle only (2) yes, motocycle or car (regardless of bicycle) (0) no	|__|
Energy	**ASK:** What is the main source of energy for lighting in the residence? (1) Firewood or batteries (2) LGP, oil/paraffin/kerosene, or candles (3) other (3) Electricity, generator, or solar panel	
Iron	**ASK:** Does the household have a non-electric or electric clothes iron? (1) yes (0) no	
fridge	**ASK:** does this HH own a working freezer? (1) yes (0) no	|__|
Clock	**ASK:** does the household have a clock (mobile phone, wall, wrist, or pocket)? (1) yes (0) no	
Radio	**ASK:** does the household have a radio, stereo system, or cassette player? (1) yes, radio only (2) yes, stereo system or cassette player (regardless of radio) (0) no	
sofa	**ASK/OBSERVE:** does this HH own a sofa? (1) yes (0) no	
kitchen	**ASK/OBSERVE:** where is the kitchen located? (1) inside house (0) outside house or not connected to the main house	|__|
hhfloor	**ASK/OBSERVE:** What is the main material of the floor of the residence (excluding kitchen and bathrooms)? (1) Uncovered, or other	|__|

	(2) wood/parquet, marble/granite, cement, or mosaic/tile		
Hhwalls	**ASK:** What is the main material of the walls of the residence? (1) Reeds/sticks/bamboo/palm, wood or metal sheets, tin/cardboard/paper/sacks or other (2) Adobe locks, wattle and daub, cement blocks, or bricks		
wingrate	**ASK/OBSERVE:** does the HH have grated windows? (1) yes (0) no		|__|
doorgrate	**ASK/OBSERVE:** does the house have a grated door? (1) yes (0) no		|__|
drinkwat	Onde é que a sua família **normalmente** busca **água para beber?** *Se a família busca água para beber de fontes múltiplas, procura saber qual é* ***a fonte mais utilizada*** *durante o ano.* *Não permitidas respostas múltiplas* *Utilize as folhas com figuras para acertar o tipo de fonte.*	1. ..... orneira dentro da casa 2. ..... orneira no composto 3. ..... orneira pública / fontanário 4. ..... uro 5. ..... oço protegido 6. ..... oço não protegido 7. ..... ollected rainwater 8. ..... utro 99. Não querem dizer ou não sabem	1 2 3 4 5 6 7 8 99
hhsan	ASK and OBSERVE: Qual é a latrina ou sistema mais utilizada pelos membros da família?	1. ..... Flush/pour flush toilet to piped sewer system 2. ..... F lush/pour toilet to onsite, underground 3. ..... lush/pour toilet to onsite, aboveground 4. ..... it latrine with concrete slab (not pour flush) 5. ..... it latrine without slab (not pour flush)	1 2 3 4 5 6 7 8 9 10 99

		6. ..... ucket 7. ..... pen defecation (no facilities) 8. ..... utro 99. Não querem dizer ou não sabem	
Drophole	ASK/OBSERVE: Is the drop hole covered?	(1) Yes (0) No	
Tankseal	ASK/OBSERVE: Is the underground tank/hole sealed?	(1) Yes (0) No (99) I don't know	
Ventpipe	ASK/OBSERVE: Is there a covered ventilation pipe?	(1) Yes (0) No	
Masonry	ASK/OBSERVE: Is there a masonry or tile slab, or pedestal?	(1) Yes (0) No	
Child questions for children under 4 years - ask every child who is under 4 years in the household.			
ChildID	Child ID #		
respmom	Is the respondent the child's mother?	(1) Yes (skip to careeedu question) (0) No	
mompresent	Is the child's mother alive and living in the household?	(1) yes (0) no	
resprelat	If respondmom= no; ASK: What is the respondent's relationship to the child?	(1) father (2) female relative (3) male relative (5) other female caregiver (6) other male caregiver	
careedu	ASK: what education did caregiver of the child under 4 receive? (if several caregivers record highest education level)	(0) None (1) Some primary (2) Primary completed (3) Some secondary (4) Secondary completed (5) Some technical training (6)Technical training completed (7)Some higher education (8) Higher education completed (88) Refused question (99) Don't know	
childname	What is the child's name?		
birthdate	ASK: what is the child's birthdate?	Format: DD/MM/YYYY	

	CHECK: vaccination card, postnatal card details Add 01/01/2000 if date not available.		
Age	IF birthdate not available: ASK: in what year and month was the child born If month not available, mark month as 99 If year not available, exclude unless obviously under 5 years	Format: MM/YYYY	
Breastfed	Is the child currently breastfeeding?	(1) Yes, exclusively (2) Yes, in addition to other foods and liquids (3) No (4) Don't know	
diaper	ASK: is this child still in diapers? If the child wears diapers sometimes, mark as yes.	(1) yes, disposable (2) yes, cloth (0) no *(99) don't know*	
diapdisp	IF YES, uses disposable diapers, ASK: where is the diaper disposed when child is changed?	(1) Latrine (2) Rubbish heap (3) Soak pit (4) other *(99) don't know*	
childdef	IF NO, does not wear diapers, ASK: where does the child defecate?	(1) Latrine (2) Potty (3) Floor (4) other *(99) don't know*	
Crèche	ASK: how many days per week is this child going to a crèche?	Enter Number of days (99) don't know	
diarrhoea	Did this child have diarrhoea at any time in the last 7 days? Diarrhea is considered to be the passage of >=3 loose or liquid stools in a 24 hour period or any stool with blood	(1) Yes (0) No *(99) don't know*	
Diabout	One the worst day of this episode, how many bouts	Enter number of days 0 - 7	

	of diarrhoea did this child have? Enter 99 if respondent does not know.		
diadays	If more than 3 bouts: For how many days did this child have 3 or more bouts?	Enter Number of days (99) don't know	
diaeat	If diarrhoea= yes During this time, did this child refuse to eat on one or more days? If YES: On how many days?	Enter number of days (0) No refusal to eat *(99) don't know*	
diafev	If diarrhoea= yes During this time, did this child have fever on one or more days? If YES: On how many days?	Enter number of days (0) No fever *(99) don't know*	
diablood	If diarrhoea= yes During this time, did this child ever have blood in stools?	(1) Yes (0) No (99) Don't know	
treat	Procurou conselhos ou tratamento para a diarreia?	(1) Yes (0) No (99) Don't know	
treatsource	If treat = yes Onde procurou conselho ou tratamento? Em algum outro lugar? Anote todas as respostas	Sector publico (1) unidade sanitaria (2) brigada movel (3) outro publico Sector privado (4) clínica (5) farmacia (6)medico (7) outro privado **Outra Fonte** (8) dumba nengue (9)medico tradicional (10) pessoal de saude do bairro (11) outro (99) don't know	

treattime	How many days after the illness began did you first seek advice or treatment?	(1) mesmo dia (2) dia segunte (3) after 3 or more days (4) don't know	
vomit	In the last 7 days, did this child vomit on one or more days? If YES: On how many days did this child have vomiting?	Enter number of days or: (0) No vomiting (99) don't know	
CHRONIC ABDOMINAL SYMPTOMS AND WEIGHT / HEIGHT MEASURES FOR CHILDREN LESS THAN 4 YEARS OLD ***First ask questions, then ask to be allowed to measure weight and height***			
chronpain	**In the last week did this child have abdominal pain?** *If YES: on how many days out of the past week?*	Enter number of days or: (0) No pain (99) don't know	
chronpainloc	If abdominal pain: Was this pain in a particular place of the belly or everywhere?	(0) local (1) everywhere (99) don't know	
paineat	During this time, did this child refuse to eat on one or more days because of abdominal pain? If YES: On how many days?	Enter number of days or: (0) No refusal to eat (99) don't know	
weight	For children less tan 24 months, use tared weight. For children > 24 months, measure child weight alone.	Enter weight in (kg)	
height	For children less than 24 months, measure recumbent length. For children older than 24 months, measure standing height. All measurements should	Enter height in (cm)	

	be recorded in centimeters (cm).		

.

Table C3: 12-month and 24-month Compound survey

Question text (Eng)	**Question Text (Port)**	**Answer text (Eng)**	**Answer text (Port)**
N/A	N/A	Compound survey v.1.	Compound survey v.1.
N/A	N/A	MapSanStudy	MapSanStudy
N/A	N/A	Final	Final
		Draft	Draft
N/A	N/A	unique identifier for survey	unique identifier for survey
N/A	N/A	MM/DD/YYYY H:MM	MM/DD/YYYY H:MM
N/A	N/A	MM/DD/YYYY H:MM	MM/DD/YYYY H:MM
Enter enumerator ID #	ID do inquiridor	Isabel	Isabel
		Sergio	Sergio
		Carolina	Carolina
		Maria Celina	Maria Celina
		Olimpio	Olimpio
		Zaida	Zaida
N/A	N/A	MM/DD/YYYY H:MM	MM/DD/YYYY H:MM

Enter the Bairro ID	ID do Bairro (nome oficial)	Aeroporto A	Aeroporto A
		Aeroporto B	Aeroporto B
		Chamanculo A	Chamanculo A
		Chamanculo B	Chamanculo B
		Chamanculo C	Chamanculo C
		Malanga	Malanga
		Maxaquene A	Maxaquene A
		Maxaquene B	Maxaquene B
		Maxaquene C	Maxaquene C
		Maxaquene D	Maxaquene D
		Minkadjuine	Minkadjuine
		Munhuana	Munhuana
		Unidade 7	Unidade 7
		Urbanizacao	Urbanizacao
		Xipamanine	Xipamanine
Enter the Quarteirao number	N° do Quarteirão (oficial)	N/A	N/A
Is this an intervention or control compound?	Este é um composto de intervenção ou de controlo	Intervention	Intervenção
		control	controlo

What type of latrine did the compound receive?	Que tipo de latrina o composto recebeu?	Communal sanitation block	Bloco sanitário
		shared latrine	Latrina partilhada
Was the compound previously part of the study?	O composto foi inscrito anteriormente no estudo?	Yes Written consent was obtained at one or more households in the compound	Sim Consentimento escrito foi obtido em uma ou mais casas no composto?
		No Written was NOT obtained from any households in the compound	Não Consentimento escrito não foi obtido em uma ou mais casas no composto
Who is the survey respondent?	Quem é o entrevistado?	Head of the compound	Chefe do Composto
		Spouse of head of compound	Esposa (o) do (a) Chefe do Composto
		Other (please specify)	Outro (especifique)
resp_Other_please_specify_		N/A	N/A
respNum	Qual é o número de telefone do entrevistado?	8########	8########

What is the name of the current head of the compound?	Qual é o nome do actual chefe do composto?	name	name
What is the phone number of the current head of the compound?	Qual é o número de telefone do actual chefe do composto?	8########	8########
		TRUE	TRUE
Has the head of the compound changed in the past year?	O chefe do composto foi substituído no último ano?	Yes	Sim
		No	Nao
What is the name of the old head of the compound?	Qual é o nome do antigo chefe do composto?	name	name
		TRUE	TRUE
Enter the compound ID assigned at baseline.	Insira o ID do composto atribuído no estudo de base	numeric	numeric
N/A	N/A	MM/DD/YYYY H:MM	MM/DD/YYYY H:MM
N/A	N/A	GPS	GPS
N/A	N/A	GPS	GPS
N/A	N/A	GPS	GPS
N/A	N/A	GPS	GPS
How many households are in this compound?	Quantos agregados familiares existem neste composto?	##	##

How many people are living in this compound right now?	Quantas pessoas vivem neste composto actualmente?	##	##
Is there a child less than 5 years old living in this compound right now?	Existe alguma criança com menos de 5 anos (60 meses) que vive neste composto actualmente?	Yes	Sim
		No	Nao
How many children <5 years old live in this compound right now?	Quantas crianças com menos de 5 anos (60 meses) vivem neste composto actualmente?	##	##
Since we last visited, has your compound received any education or training related to hygiene promotion or sanitary practices?	Desde a nossa última visita, o composto recebeu educação ou formação em matéria de higiene ou práticas sanitárias?	Yes	Sim
		No	Nao
		TRUE	TRUE
Who provided the education or training?	Quem facilitou o treinamento?	The local CBO	Uma organização baseada na comunidade
		Someone from WSUP	Alguém da WSUP
		Someone from	Alguém do MISAU

		MISAU	
		Other (please specify)	Outro (especifique)
		N/A	N/A
		TRUE	TRUE
Did you compound receive deworming medication from our study team following our first visit last year?	O seu composto receber medicação de desparasitação da nossa equipa após a nossa primeira visita no ano passado?	Yes	Sim
		No	Nao
		TRUE	TRUE
Other than the medication administered by our study team, has anyone visited your compound to administer additional deworming medication in the past year?	Para além da medicação administrada pelo estudo, uma outra pessoas ou instituição visitou o composto e deu medicação para desparasitação no ano passado?	Yes, someone from the Ministry of Health	Sim, alguém do MISAU
		Yes, someone from a different organization	Sim, alguém de uma organização diferente

		No, no one has visited	Não, ninguém visitou
		TRUE	TRUE
About how long ago did your compound receive deworming medication?	A quanto tempo o composto recebeu a medicação de desparasitação?	within the last week (0-7 days)	Dentro da semana passada (0 -7 dias)
		within the last month (8-30 days)	Dentro do mês passado (8-30 dias)
		within the last 3 months (31 - 90 days)	Dentro dos últimos 3 meses (31-90 dias)
		within the last 6 months (91 - 180 days)	Dentro dos últimos 6 meses (91-180 dias)
		longer than 6 months ago (180+ days)	A mais de 6 meses (180+ dias)
		TRUE	TRUE
Take photos of the compound grounds from the entrance of the compound.	Tire fotos do composto (espaço partilhado) a partir do centro do composto	N/A	N/A

Stand in the center of the compound and click "current location"	Posicione-se no centro do composto e clique "localização actual"	GPS	GPS
		GPS	GPS
		GPS	GPS
		GPS	GPS
Is there electricity in the compound that normally functions?	O composto tem sempre acesso a electricidade?	Yes	Sim
		No	Nao
OBSERVE: Is there standing water in the compound?	OBSERVE: Existe água estagnada no composto?	Yes	Sim
		No	Nao
Take a photo of the standing water.	Tire uma foto da água estagnada	N/A	N/A
OBSERVE: Are there feces or or used diapers on the ground or in a rubbish heap?	OBSERVE: existem fezes ou fraldas usadas no chão ou num monte de lixo?	Yes	Sim
		No	Nao
Take a photo of the feces or diapers.	Tire uma foto das fezes ou fraldas	N/A	N/A
Does the compound flood in the rainy season?	O composto fica inundado na época chuvosa?	Yes	Sim

		No	Nao
Has the compound flooded in the past month?	O composto ficou inundado no último mês?	Yes	Sim
		No	Nao
If the compound is currently flooded, take a photo.	Tire uma foto se o composto estiver inundado no momento	N/A	N/A
How does your compound get rid of rubbish (solid waste)?	Como o seu composto deita o lixo (resíduos sólidos)?	Municipality removes it	removido pelo município
		Bury it	enterrado
		Burn it	Queimado
		Move it outside of the compound	Removido para foram do composto
		TRUE	TRUE
What animals are regularly present in the compound? Select all that apply.	Que animais estão regularmente presentes no composto? Selecione todas as opções aplicáveis.	None	Nenhum
		Dog	Cão
		Cat	Gato
		Chicken/duck	Galinhas/patos
		Other (please specify)	Outro (especifique)
		N/A	N/A

How many dogs are regularly present in the compound?	Quantos cães estão normalmente presentes no composto?	#	#
How many cats are regularly present in the compound?	Quantos gatos estão normalmente presentes no composto?	#	#
How many chickens or ducks are regularly present in the compound?	Quantos patos e/ou galinhas estão normalmente presentes no composto?	#	#
How many "other" animals are regularly present in the compound?	Quantos 'outros" animais estão normalmente presentes no composto?	#	#
Do the animals defecate in the compound area?	Os animais defecam no pátio do composto?	Yes, the animals defecate anywhere within the compound	Sim, os animais defecam em qualquer lugar dentro do composto
		Yes, the animals defecate in one location inside the compound (e.g. pen)	Sim, os animais defecam em um local no interior do composto (ex. na capoeira)
		No, the animals defecate outside of the compound	Não, os animais defecam fora do composto
How many water points are in the compound?	Quantas fontes de água existem dentro do composto?	#	#
Take a photo of each waterpoint.	Tire uma foto de cada fonte de água	N/A	N/A

		GPS	GPS
		GPS	GPS
		GPS	GPS
		GPS	GPS
How many latrines/dropholes are in the compound?	Quantas latrinas existem no composto?	#	#
In the past 12 months, how much money did your compound spend on the construction and upkeep of your current latrine?	Nos últimos 12 meses, quanto dinheiro o composto gastou para construção e manutenção da actual latrina?	#	#
		TRUE	TRUE
How much money did your compound contribute upfront to help pay for the construction costs?	Quanto dinheiro o seu composto contribuiu inicialmente para ajudar na construção da vossa actual latrina?	#	#
		TRUE	TRUE
How much money does your compound pay towards the latrine each month?	Quanto dinheiro o seu composto contribui mensalmente para despesas relacionadas com a latrina?	#	#

		TRUE	TRUE
When the latrine pit becomes full, how will you empty it?	Quando a latrina ficar cheia, como vão esvaziar?	Formal large business (e.g. vacuum tanker)	Negócio formal grande (ex. Tanque a vácuo)
		Formal small business using manual or automated tool (e.g. pump)	Negócio formal pequeno com recurso a equipamento manual ou automatizado
		Members of the compound or informal manual emptier	Membros do composto ou esvaziador individual informal
		Will not empty. Will cover and dig a new pit.	Não será esvaziado. Irão cobrir a fossa/ buraco e cavar um novo.
		Not yet emptied	Ainda nao foi esvaziada
		Other (please specify)	Outro (especifique)

		N/A	N/A
How will you dispose of the latrine contents?	Como irá desfazer-se dos resíduos retirados da latrina?	Bury it in the compound	Enterrar no composto
		Put it in the rubbish pile	Colocar no monte lixo no composto
		Remove it from the compound	Remover do composto
		Other (please specify)	Outro (especifique)
		N/A	N/A
Take a photo of the interior and exterior of each latrine in the compound.	Tire fotos do interior e exterior de cada latrina existente no composto	N/A	N/A
		GPS	GPS
		GPS	GPS
		GPS	GPS
		GPS	GPS

Stand outside of the latrine and click "current location" to record GPS points of the latrine.	Posicione-se fora da latrina e clique "localização actual" para gravar as coordenadas de GPS da latrina.	GPS	GPS
		GPS	GPS
		GPS	GPS
		GPS	GPS
OBSERVE: Is the latrine inside the compound or outside of the compound?	Existe uma latrina dentro do composto ou fora do composto?	Inside	Dentro
		Outside	Fora
		Not Applicable	Não aplicável
OBSERVE: Is the latrine located on higher ground than the rest of the compound?	OBSERVE: a latrina está localizada em um ponto superior em relação as demais infraestruturas existentes no composto?	Yes	Sim
		No	Nao

		Not Applicable	Não aplicável
OBSERVE: Does the latrine have stone walls?	OBSERVE: a latrina têm paredes de pedra/blocos de cimento?	Yes, on all cabins	Sim, em todas as cabines
		Yes, on some cabins	Sim, em algumas cabines
		No	Nao
		Not Applicable	Não aplicável
OBSERVE: Is the drophole covered?	OBSERVE: o latrina/ buraco esta coberto?	Yes	Sim
		No	Nao
		Not Applicable	Não aplicável
OBSERVE: Is the drophole clogged?	OBSERVE: a latrina/buraco está obstruído?	Yes	Sim
		No	Nao
		Not Applicable	Não aplicável
OBSERVE: Does the latrine have a ventilation pipe?	OBSERVE: a latrina possui um tubo de ventilação?	Yes	Sim

		No	Nao
		Not Applicable	Não aplicável
Is the ventilation pipe covered?	O tubo de ventilação está coberto?	Yes	Sim
		No	Nao
OBSERVE: Is the underground tank/hole sealed?	OBSERVE : O tanque subterrâneo / buraco está selado ?	Yes	Sim
		No	Nao
		Not Applicable	Não aplicável
OBSERVE: Is there a masonry or tile slab, or pedestal?	OBSERVE: Existe uma laje de alvenaria, azuleijo ou pedestral?	Yes	Sim
		No	Nao
		Not Applicable	Não aplicável

OBSERVE: Is the pedestal intact?	OBSERVE: o pedestral está intacto?	Yes	Sim
		No	Nao
		Not Applicable	Não aplicável
ASK: Is the pit full? (Is the compound still using the pit for defecation or is it no longer in use?)	Pergunte: a fossa esta cheia? (O composto ainda usa a latrina para defecar ou a latrina não esta em uso?)	Yes	Sim
		No	Nao
		Not Applicable	Não aplicável
		Don't Know	Não sabe
Measurement 1 - pit opening to sludge (Measure the distance from the pit opening to the sludge using the laser tape measure.)	Medição 1 - buraco da latrina (Medir a distância do buraco da latrina e o nível das fezes, usando a fita métrica a laser .)	###	###
Measurement #2 for pit fullness. Top of pedestal/tire/etc to ground. (If no second measurement is needed, enter 0)	Medição 2 - medição da distância do topo do pedestal/pneu para o solo (início do buraco da latrina). (Se não for necessária uma segunda medida, digite 0)	###	###
Take a photo of the pit.	Tire uma foto da fossa	N/A	N/A

OBSERVE: Is there open wastewater near the latrines or leaking from the latrines?	OBSERVE: Existem águas residuais próximo ou a vasar da latrina?	Yes	Sim
		No	Nao
Take a photo of the standing or leaking wastewater.	Tire uma foto das água residuais próximas a latrina ou a vazar da mesma	N/A	N/A
OBSERVE: Is there a door on the latrine that functions? (Can the door be opened and closed easily)	OBSERVE: existe uma porta em funcionamento na latrina? (A porta pode ser aberta e fechada com facilidade?)	Yes	Sim
		No	Nao
		Not Applicable	Não aplicável
During the day, is the latrine usually locked on the outside?	Durante o dia, a latrina fica normalmente tracada por fora?	Yes	Sim
		No	Nao
		Not Applicable	Não aplicável
At night, is the latrine usually locked on the outside?	Durante a noite, a latrina fica normalmente trancada por fora?	Yes	Sim

		No	Nao
		Not Applicable	Não aplicável
OBSERVE: Was the latrine locked when you entered the compound?	OBSERVE: A latrina estava trancada quando iniciou a visita ao composto?	Yes	Sim
		No	Nao
		Not Applicable	Não aplicável
Do all of the households in this compound have a key to the latrine?	Todos os agregados familiares do composto possuem as chaves da latrina?	Yes	Sim
		No	Nao
		Not Applicable	Não aplicável
OBSERVE: Is there a lock on the inside of the cabin that the user can use for privacy?	OBSERVE: existe uma tranca por dentro do compartimento da latrina que possa ser usada para garantir privacidade?	Yes on all cabins	Sim, em todas as cabines
		Yes on some cabins	Sim, em algumas cabines
		No	Nao

		Not Applicable	Não aplicável
OBSERVE: Is there a handwashing station at or near the latrine?	OBSERVE: existe uma estação para a lavagem das mãos dentro ou próximo ao espaço da latrina?	Yes, built into the latrine	Sim, construído junto ao espaço da latrina
		Yes, near the latrine	Sim, próximo a latrina
		No	Nao
OBSERVE: Is there access to water at the handwashing station?	OBSERVE: existe acesso a água na estação de lavagem das mãos?	Yes, there is a water supply connected to handwashing station	Sim, existe uma conexão de água canalizada na estação de lavagem das mãos
		Yes, water bucket or container near station with water present	Sim, existe um balde/recipiente com água próximo a estação de lavagem das mãos
		No.	Não
OBSERVE: Is there soap present at the handwashing station?	OBSERVE: Existe sabão na estação de lavagem das mãos?	Yes	Sim
		No	Nao
OBSERVE: Does the latrine appear to be in use?	OBSERVE: a latrina aparenta estar em uso?	Yes	Sim

		No	Nao
		Not Applicable	Não aplicável
OBSERVE: Is the floor of the latrine wet?	OBSERVE: o chão da latrina está molhando?	Yes	Sim
		No	Nao
		Not Applicable	Não aplicável
Is the latrine seat dusty?	OBSERVE: acento da latrina está empoeirado?	Yes	Sim
		No	Nao
		Not Applicable	Não aplicável
OBSERVE: Does the latrine appear clean?	OBSERVE: a latrina aparenta estar limpa?	Yes	Sim
		No	Nao
		Not Applicable	Não aplicável
Does anyone who does not live in the compound regularly use the latrine in the compound?	Alguém que não vive no composto usa regularmente a latrina?	Yes	Sim
		No	Nao

How many people from outside the compound regularly use the latrine?	Quantas pessoas de fora do composto normalmente usam a latrina?	##	##
Are there any latrines not built by WSUP on the compound grounds?	Existe alguma latrina não construída pela WSUP dentro composto?	Yes	Sim
		No	Nao
Is the non-WSUP latrine(s) in use?	A latrina não construída pela WSUP está em uso?	Yes	Sim
		No	Nao
Do all families in the compound use all of the latrines?	Todas as famílias no composto usam todas as latrinas?	Yes	Sim
		No	Nao
N/A	N/A	MM/DD/YYYY H:MM	MM/DD/YYYY H:MM

Table C4: 12- and 24-month Household level survey

Question text (Eng)	Question Text (Port)	Answer text (Eng)	Answer text (Port)
N/A	N/A	Compound survey v.1.	Compound survey v.1.
N/A	N/A	MapSanStudy	MapSanStudy
N/A	N/A	Final	Final
		Draft	Draft
N/A	N/A	unique identifier for survey	unique identifier for survey
N/A	N/A	MM/DD/YYYY H:MM	MM/DD/YYYY H:MM
N/A	N/A	MM/DD/YYYY H:MM	MM/DD/YYYY H:MM
Field worker ID	ID do Inquiridor	Isabel	Isabel
		Sergio	Sergio
		Carolina	Carolina
		Olimpio	Olimpio
		Zaida	Zaida
		Maria Celina	Maria Celina

N/A	N/A	MM/DD/YYYY H:MM	MM/DD/YYYY H:MM
Bairros ID (official)	ID do Bairro (oficial)	Aeroporto A	Aeroporto A
		Aeroporto B	Aeroporto B
		Chamanculo A	Chamanculo A
		Chamanculo B	Chamanculo B
		Chamanculo C	Chamanculo C
		Malanga	Malanga
		Maxaquene A	Maxaquene A
		Maxaquene B	Maxaquene B
		Maxaquene C	Maxaquene C
		Maxaquene D	Maxaquene D
		Minkadjuine	Minkadjuine
		Munhuana	Munhuana
		Unidade 7	Unidade 7
		Urbanizacao	Urbanizacao
		Xipamanine	Xipamanine

Quarteirao ID (official)	Número do Quarteirão (oficial)	N/A	N/A
Compound number (given by study)	Número do composto (atribuído pelo Estudo)	####	####
Is this an intervention or control compound?	Este é um composto de intervenção ou de controlo	Intervention	Intervenção
		Control	Controlo
How many people live in this household right now?	Quantas pessoas vivem actualmente neste agregado familiar?	##	##
Was this household previously part of the study?	Este agregado familiar fez parte do estudo de base?	Yes (received written consent from Household at baseline)	Sim (Consentimento escrito do agregado familiar)
		No (Did not receive written consent from household at baseline)	Não (Não foi obtido consentimento escrito do agregado)
Is there a child less than 5 years old living in this household right now?	Existe alguma criança menor de 5 anos (60 meses) a viver actualmente neste agregado familiar?	Yes	Sim
		No. Stop survey here.	Não (TERMINE AQUI)
What was the HH ID assigned at baseline?	N/A	##	##

Enter the household ID assigned at baseline.	Insira o ID do AF atribuído no estudo de base.	##	##
Enter a new HH ID. If the To differentiate the newly enrolled HHs from HHs enrolled at baseline, HH IDs for newly enrolled HHs will start at 50 and go up sequentially within a compound.	Insira um novo ID do AF. Para diferenciar um novo AF do AF visitado no estudo de base, o novo AF deve iniciar pelo número 50 e aumentar sequencialmente dentro do composto.	##	##
Assign the household a HH ID	Atribua um ID ao agregado familiar	##	##
Have you gone over the verbal consent information sheet with the respondent and answered any study related questions? (QUESTION TEXT FOR PREVIOUSLY ENROLLED HOUSEHOLDS)	Percorreu o formulário do consentimento informado verbal com o entrevistado (a) e respondeu a qualquer dúvida que o mesmo tenha apresentado?	Yes	Sim
Have you gone over the participant information sheet and consent form with the respondent and answered any questions? (QUESTION TEXT FOR NEWLY ENROLLED HOUSEHOLDS)	Verificou o formulário de informação do participante e o consentimento juntamente com o mesmo. Respondeu qualquer dúvida ou questão relacionada com o estudo?	No. Do not continue with survey until this is complete.	Não. Não continue com o questionário até completar esta etapa.

Does the household agree to remain in the study?	O agregado familiar concorda em continuar no estudo?	Yes	Sim
Has the respondent filled out the consent form?	O entrevistado (a) preencheu o formulário de consentimento?	No. Stop here.	Não (TERMINE AQUI)
What is the respondent's name?	Qual é o nome do entrevistado?	Respondent's name	Respondent's name
What is the respondent's telephone number?	Qual é o número de contacto do entrevistado?	8########	8########
		N/A	N/A
What is the head of household's name?	Qual é o nome do chefe do agregado familiar?	name	name
What is the head of the household's telephone number?	Qual é o número de telefone do chefe do agregado?	8########	8########
		TRUE	TRUE
		TRUE	TRUE
Stand at the front door of the household and record GPS points by pressing "current location"	Posicione-se na porta de entrada do agregado e grave as coordenadas de GPS ao clicar em "localização actual"	GPS	GPS
		GPS	GPS

		GPS	GPS
		GPS	GPS
Take a photo of the interior and exterior of the household.	Tire foto do interior e do exterior da casa do AF	N/A	N/A
Who is the respondent? (If possible, the mother or female caregiver should be the respondent)	Quem é o(a) entrevistado(a)? (Se possível, entreviste a mãe ou mulher cuidadora da criança.)	Male head of household	Homem chefe do agregado
		Female head of household	Mulher chefe do agregado
		female caregiver	Mulher cuidadora
		male caregiver	Homem cuidador
		Other representative (please specify)	Outro (especifique)
		role of respondent	role of respondent
What religion is practiced in this household?	Qual é a religião praticada pelo agregado?	Christianity (Catholic or Protestant)	Cristianismo (Católica ou protestante)
		Islam	Islão
		Prefer not to answer	Prefere não responder
		Other (please specify)	Outro (especifique)
		N/A	N/A
What education did the head of the household receive?	Qual é o nível de escolaridade do chefe do agregado?	some primary school	Ensino primário incompleto

		primary completed	Ensino primário completo
		some secondary	Ensino secundário incompleto
		secondary completed	Ensino secundário completo
		some technical training	Ensino técnico incompleto
		technical training completed	Ensino técnico completo
		some higher education	Ensino superior incompleto
		higher education completed	Ensino superior completo
		TRUE	TRUE
What is the household's relationship with the other members or households of the compound?	Qual é a relação do AF com os demais membros ou agregados do composto?	Family	Família
		Friends	Amigos
		Other established relationship	Outro tipo de relação estável

		No relationship	Nenhuma relação
		N/A	N/A
Do you own or rent your property?	A casa é arrendada ou é propriedade do agregado?	Own	Própria
		Rent	Arrendada
In the month, how much did your household spend on food including things such as rice/xima, meat, fruits, vegetales, and cooking oils. Include the value of any food that produced and consumed by the household and exclude alcohol, tobacco, and restaurant meals.	Num mês quanto é que o seu agregado familiar gastou em comida, incluindo itens como arroz/xima, carne, frutas, vegetais e óleo de cozinha. Incluí o valor de qualquer produto alimentar produzido e consumido pelo agregado e excluí álcool, Tabaco e refeições compradas em restaurantes/barracas	###	###
How much money do you spend just on rent each month?	Quanto gasta mensalmente com a renda de casa?	###	###
In the past month, how much did your household spend on housing, gas, electricity, water, telephone, and heating fuel?	No último mês quanto é que o seu agregado familiar gastou em dispesas de renda da casa, gás, electricidade, carvão, água, telefone	###	###
How much money does your household spend on education fees and supplies in a typical month?	Num mês típico quanto é que o seu agregado familiar gastou em materiais e propinas escolares	###	###
In the past month, how much money did your household spend on healthcare costs,	No último mês quanto é que o seu agregado familiar gastou em cuidados	###	###

excluding any insurance reimbursements?	de saúde		
In the past month, how much money did your household spend on all other goods and services? (E.g. restaurant meals, transportation costs, events (wedding, funerals)	No último mês quanto é que o seu agregado familiar gastou em todos outros bens e serviços (Por exemplo. refeições em restaurantes, os custos de transporte , eventos (casamento, funerais)	###	###
In the past month, how much money did your household spend in total? (Ask respondents to consider all costs (e.g. food, rent, utilities, cell phones, etc). Respondent can estimate.)	No último mês quanto é que o seu agregado gastou no total? (Peça ao entrevistado(a) para considerar todos os custos (ex. Alimentação, renda, crédito, etc). O entrevistado (a) pode estimar)	###	###
How many children less than 5 years old live in your household?	Quantas crianças menores de 5 anos vivem no agregado?	###	###
How many bedrooms are in your house?	Quantos quartos existem no agregado?	###	###
How many beds does this household have (single, double, bunkbeds, or for children)?	Quantas camas existem no agregado (solteira, dupla, beliche, ou berço)?	None	Nenhuma
		One	Uma
		Two or more	Duas ou mais
Does the household own a working bicycle, motorcycle, or car?	O agregado possuí uma bicicleta, motorizada ou carro em funcionamento?	Bicycle	bicicleta

		Motorcycle	motocicleta
		Car	Carro
		None	Nenhuma
What is the main source of energy for lighting in the residence?	Qual é a principal fonte de energia para iluminação da residência?	Firewood or batteries	Lenha ou baterias/pilha
		LGP, oil/paraffin/kerosene or candles	LGP, óleo/parafina/querosene, ou velas
		Electricity, generator, or solar panel	Electricidade, gerador, ou painel solar
		Other (please specify)	Outro (especifique)
		N/A	N/A
Does the household have a non-electric or electric clothes iron?	Existe no agregado um ferro de engomar eléctrico ou não eléctrico?	Yes	Sim
		No	Não
Does the household own a working freezer?	O agregado possuí uma geleira em funcionamento?	Yes	Sim
		No	Não
Does the household own a working clock? (E.g. mobile phone, wall clock, wrist watch, or pocket watch)	O agregado possuí relógio em funcionamento? (Ex. do telemóvel, de parede, do pulso, ou de bolso?)	Yes	Sim

		No	Não
Does the household own a working radio, stereo, or cassette player?	O agregado possuí rádio, leitor de CD/DVD, leitor de cassete em funcionamento?	Radio	Rádio
		Stereo	Leitor de CD/DVD
		Cassette player	Leitor de cassete
		None	Nenhuma
Does the household own a sofa?	O agregado possuí um sofá?	Yes	Sim
		No	Não
Is the kitchen located indoors (covered) or outdoors?	A cozinha encontra-se localizada no interior (coberta) ou no exterior?	Indoors	Interior
		Outdoors	Exterior
Is the kitchen attached to the house or is it in a communal area in the compound?	A cozinha está localizada dentro do espaço da residência ou encontra-se em uma área comum dentro do composto?	Attached to household	Parte do agregado
		In communal area	Na área comum
What is the main material of the floor of hte residence?	Qual é o principal material do piso da residência? (excluindo cozinha e casas de banho)?	uncovered	Descoberto
		wood/parquet, marble/granite, cement, mosaic/tile	madeira/parquet/cimento/mozaico

		Other (please specify)	Outro (especifique)
		N/A	N/A
What is the main material of the wall of the house?	Qual é o principal material das paredes da casa?	reeds/sticks/bamboo/palm	Palhetas/paus/bambus/folhas de palmeira
		wood or metal sheets	madeira ou metal
		tin/cardboard/paper/sacks	papelão/papel/sacos
		Adobe locks	Adobe
		wattle and daub	Pau a pique
		cement blocks or bricks	Blocos de cimento ou tijolo
		Other (please specify)	Outro (especifique)
		N/A	N/A
Does the household have grated windows	O agregado tem as janelas gradeadas?	Yes	Sim
		No	Não
Does the house have a grated door?	O agregado tem as portas gradeadas?	Yes	Sim
		No	Não

Where does your household normally collect water to drink? (If more than one source is used, choose the source most often used during the year.)	Onde normalmente o agregado busca água para beber? (Caso mais de uma fonte seja usada, escolha a mais usada durante o ano.)	Tap inside the house	Torneira dentro de casa
		Tap in the compound	Torneira dentro do composto
		Public tap or fountain	Fonte de água pública ou fontanário
		protected spring	Nascente protegida
		unprotected spring	Nascente desprotegida
		collected rainwater	Água da chuva
		borehole	Furo
		Other (please specify)	Outro (especifique)
		N/A	N/A
How many hours a day is water available from your water source?	Quantas horas por dia tem acesso a água da fonte que usa?	##	##

When we visited your Household one year ago, what type of sanitation system did you use? (If intervention compound, make sure respondent understands that we are asking about sanitation BEFORE WSUP built the new latrine.)	Quando visitamos a sua casa no ano passado, que tipo de latrina ou sistema de saneamento os membros do seu agregado usava? (Se for um composto de intervenção, certifique que o entrevistado entenda que a pergunta é sobre a latrina ou sistema de saneamento que usava antes da latrina ou bloco construido pela WSUP)	Flush/pour flush toilet to piped sewer system	Sistema com água corrente ligado ao esgoto
What type of sanitation system did you use one year ago? (If intervention compound, make sure respondent understands that we are asking about sanitation BEFORE WSUP built the new latrine.) QUESTION TEXT MODIFCATION FOR NEWLY ENROLLED COMPOUNDS	A um ano atrás, que tipo de latrina ou sistema de saneamento os membros do seu agregado usava? (Se for um composto de intervenção, certifique que o entrevistado entenda que a pergunta é sobre a latrina ou sistema de saneamento que usava antes da latrina ou bloco construido pela WSUP) QUESTION TEXT MODIFICATION FOR NEWLY ENROLLED COMPOUNDS	Flush/pour flush toilet to underground pit/tank	Sistema com água corrente ligado a fossa séptica
		Flush/pour flush toilet to onsite, above ground, open pit	Sistema com água corrente para o local ou na superfície
		Pit latrine with concrete slab (not pour flush)	Latrina com laje de concreto (sistema sem descarga)

		Pit latrine without slab (not pour flush)	Latrina tradicional sem laje
		Bucket	Balde
		Bag	Plástico
		Open defecation (no facilities)	Fecalismo a céu aberto (sem instalações)
		Other (please specify)	Outro (especifique)
		N/A	N/A
Presently, what type of latrine or sanitation system do members of your family use most often?	Actualmente, que tipo de latrina ou sistema de saneamento os membros do seu agregado usam com mais frequência?	WSUP-built latrine	Latrina construída pela WSUP
		Flush/pour flush toilet to piped sewer system	Sistema com água corrente ligado ao esgoto
		Flush/pour flush toilet to underground pit/tank	Sistema com água corrente ligado a fossa séptica

		Flush/pour flush toilet to onsite, above ground, open pit	Sistema com água corrente para o local ou na superfície
		Pit latrine with concrete slab (not pour flush)	Latrina com laje de concreto (sistema sem descarga)
		Pit latrine without slab (not pour flush)	Latrina tradicional sem laje
		Bucket	Balde
		Bag	Plástico
		Open defecation (no facilities)	Fecalismo a céu aberto (sem instalações)
		Other (please specify)	Outro (especifique)
		N/A	N/A
Does your facility have container to dispose of menstrual hygiene items?	No local onde esta instalada a latrina que usa existe um balde para descartar pensos higiénicos?	Yes	Sim

		No	Não
Are the latrine cabins usually locked on the outside?	O compartimento da latrina encontra-se normalmente fechado por fora?	Yes	Sim
		No	Não
		TRUE	TRUE
Do you(or your family) have a key to the latrine?	O (a) entrevistado (a) (e outros membros do agregado) tem acesso a chave da latrina?	Yes	Sim
		No	Não
Are there times when you want to use the latrine but do not have a key?	Houve/há vezes que deseja usar a latrina mas não o faz porque não tem acesso as chaves?	Yes	Sim
		No	Não

Which, if any, of the following categories of people do not regularly use the latrine?	Qual, se alguma, das categorias de pessoas não usa regularmente a latrina?	Women and girls	Mulheres e raparigas
		infants	Crianças
		People with disabilities	Pessoas com deficiência
		people with HIV/AIDS	Pessoas com HIV/SIDA
		Other (please specify)	Outro (especifique)
		None of the above (all groups of people have access)	Nenhuma das alternativas acima (todos os grupos de pessoas usam a latrina)
		N/A	N/A
On average, how many minutes do you usually wait to use the latrine?	Em média, quantos minutos normalmente espera para usar a latrina?	##	##
Does the latrine pit overflow when it rains?	A latrina transborda/enche quando chove?	Yes	Sim

		No	Não
		TRUE	TRUE
Last time the pit was emptied, who paid for the service?	Quem pagou para a latrina ser esvaziada da última vez que esta esteve cheia?	Only my household	só o meu agregado familiar
		All households that share the toilet	Todas as famílias que partilham a casa de banho
		Some of the households that share the toilet	Algumas as famílias que partilham a casa de banho
		landlord	Senhorio/ dono da casa
		Free service provided by municipality, NGO, etc	Serviço gratuito oferecido pelo município , ONG , etc
		Other (please specify)	Outro (especifique)
		N/A	N/A
Since the last time we visited your household, has anyone (unrelated to the study) come to speak with you about hygiene practices or sanitary behavior?	Alguém aproximou-se a si para falar sobre práticas de higiene ou saneamento desde a nossa última visita	Yes, the local CBO	Sim, uma organização da comunidade

In the past year, has anyone come to speak with you about hygiene practices or sanitary behavior? QUESTION TEXT MODIFICATION FOR NEWLY ENROLLED HOUSEHOLDS	Alguém aproximou-se a si para falar sobre práticas de higiene ou saneamento no ano passado, ? QUESTION TEXT MODIFICATION FOR NEWLY ENROLLED HOUSEHOLDS	Yes, someone from WSUP	Sim, alguém da WSUP
		Yes, someone from MISAU/INS	Sim, alguém do MISAU/INS
		Yes, someone from a different organization	Sim, alguém de uma organização diferente
		No	Não
Ask the respondent to confirm whether members of the household received deworming medication from a member of the MapSan team following the baseline visit? (OIimpio Zavale and a representative from MISAU administered deworming medication)	Peça ao entrevistado para confirmar se membros do seu agregado receberam medicação para a desparasitação de um membro da equipa Mapsan depois da visita do estudo de base. (OIimpio Zavale e um representante do MISAU distribuíram medicação de desparasitação)	Confirmed	Confirmado

		Unconfirmed	Não confirmado
Since the last time we visited, has anyone not involved with the MapSan trial come to the compound and administered deworming medication to household members?	Desde a nossa última visita (um ano), alguém fora da equipa Mapsan veio ao composto e administrou medicação de desparasitação aos membros do agregado?	Yes, someone from MISAU	Sim, alguém do MISAU
In the past year, has anyone not involved with the MapSan trial come to the compound and administered deworming medication to household members? QUESTION TEXT MODIFICATION FOR NEWLY ENROLLED HOUSEHOLDS	Desde a nossa última visita (um ano), alguém fora da equipa Mapsan veio ao composto e administrou medicação de desparasitação aos membros do agregado? QUESTION TEXT MODIFICATION FOR NEWLY ENROLLED COMPOUNDS	Yes, someone from a different organization	Sim, alguém de uma organização diferente
		No	Não
Was deworming medication given household members older than 5 years?	A medicação de desparasitação foi dada a membros do agregado maiores de 5 anos?	Yes	Sim
		No	Não
Was deworming medication given to children less than 5 years old?	A medicação de desparasitação foi dada a membros do agregado menores de 5	Yes	Sim

	anos?		
		No	Não
About how long ago did your compound receive deworming medication?	A quanto tempo o seu composto recebeu a medicação para a desparasitação?	within the past week (0-7 days)	Dentro da semana passada (0 -7 dias)
		within the past month (8-30 days)	Dentro do mês passado (8-30 dias)
		within the past 3 months (31 - 90 days)	Dentro dos últimos 3 meses (31-90 dias)
		within the past 6 months (90- 180 days)	Dentro dos últimos 6 meses (91-180 dias)
		Longer than 6 months ago (180+ days)	A mais de 6 meses (180+ dias)
Was the medicine given in a tablet or liquid form?	O medicamento foi dado em forma de comprimido ou em forma líquida?	Liquid	Líquido
		Tablet	Comprimido

		TRUE	TRUE
How much money did your household initially contribute to the construction of the latrine in your compound?	Quanto dinheiro o seu agregado contribuiu inicialmente para a construção da latrina existente no composto?	##	##
Does your household contribute money each month to help pay for the latrine?	O seu agregado contribui um valor monetário mensalmente para pagar para qualquer dispesa relacionada com a latrina?	Yes	Sim
		No	Não
		TRUE	TRUE
How much money does your household contribute each month?	Quanto dinheiro o seu agregado contribui mensalmente?	##	##
Is the latrine functioning right now?	A latrina está em funcionamento?	Yes	Sim
		No	Não
		TRUE	TRUE
Is there a committee assigned to cleaning and maintaining the latrine for the compound?	Existe um comité para a limpesa e manutenção da latrina?	Yes	Sim

		No	Não
		TRUE	TRUE
How often is the latrine cleaned? (Do not read the answers to the respondent. Ask the question and select the appropriate response.)	Com que regularidade a latrina é limpa? (Não leia as respostas. Faça a pergunta e seleccione a opção apropriada.)	1 time per day or more	1 ou mais vezes por dia
		1 time per week or more	1 ou mais vezes por semana
		1 time per month or more	1 ou mais vezes por mês
		less than 1 time per month	Menos de uma ves por mês
		Never	Nunca
		TRUE	TRUE
		TRUE	TRUE
When defecating at home, how often do do you use the WSUP-built latrine?	Quando defeca em casa, com que frequência usa uma latrina construída pela WSUP?	Always	Sempre
		Most of the time	Na maior parte das vezes

		Sometimes	As vezes
		Rarely	Raramente
During the last 2 days, what was your primary place of defecation?	Durante os dois últimos dias, qual foi o seu principal local para defecação?	Private latrine in my house	Latrina privada dentro de casa
		Latrine in my compound (WSUP latrine)	Latrina no composto (latrina construída pela WSUP)
		Latrine in my compound (NOT WSUP latrine)	Latrina dentro do composto (não construída pela WSUP)
		Latrine outside of my compound	Latrina fora do composto
		Bag or bucket	Plástico ou pinico/balde
		Open defecation in the compound	Fecalismo ao céu aberto no composto
		Open defecation outside of the compound	Fecalismo ao céu aberto fora do composto

What type of latrine was it?	Que tipo de latrina foi?	Pour flush to underground pit/tank	Sistema com água corrente ligado a fossa séptica
		Pour flush to sewer system	Sistema com água corrente ligado ao esgoto
		Pour flush to elsewhere (above ground, open)	Sistema com água corrente para o local ou na superfície
		Pit latrine with concrete slab (not pour flush)	Latrina com laje de concreto (sistema sem descarga)
		Pit latrine without concrete slab (not pour flush)	Latrina tradicional sem laje
What type of latrine did you use outside of your compound?	Que tipo de latrina usa fora do seu composto?	WSUP buit latrine	Latrina construída pela WSUP
		Pour flush to underground pit/tank	Sistema com água corrente ligado a fossa séptica

		Pour flush to sewer system	Sistema com água corrente ligado ao esgoto
		Pour flush to elsewhere (above ground, open)	Sistema com água corrente para o local ou na superfície
		Pit latrine with concrete slab (not pour flush)	Latrina com laje de concreto (sistema sem descarga)
		Pit latrine without concrete slab (not pour flush)	Latrina tradicional sem laje
Was the latrine outside of the compound private, shared, or public?	A latrina fora do composto era privada, partilhada, ou pública	Private (in a private home or business)	Privada (Em uma casa ou estabelecimento)
		Shared (shared by a known group of people)	Partilhada (Partilhada por um grupo de pessoas conhecidas)
		Public (used by a large group of unknown people)	Público (Usada por um grupo grande de pessoas desconhecidas)
During the last 2 days, did you defecate anywhere else?	Durante os últimos 2 dias, defecou em algum outro local?	Private latrine in my house	Latrina privada dentro de casa
		Latrine in my compound (WSUP latrine)	Latrina no composto (latrina construída pela WSUP)

		Latrine in my compound (not WSUP latrine)	Latrina dentro do composto (não construída pela WSUP)
		Latrine outside of my compound	Latrina fora do composto
		Into bag or bucket	Plástico ou penico/balde
		Open defecation in the compound	Fecalismo ao céu aberto no composto
		Open defecation outside of the compound	Fecalismo ao céu aberto fora do composto
		No	Não
What type of latrine was it?	Que tipo de latrina foi?	Pour flush to undeground pit/tank	Com descarga para uma fossa ou tanque séptico
		Pour flush to underground sewer system	Com descarga para o Sistema de esgoto
		Pour flush to elsewhere (above	Com descarga para um outro local (acima do solo)

		ground)	
		Pit latrine with concrete slab (not pour flush)	Latrina com laje de concreto (sistema sem descarga)
		Pit latrine without concrete slab (not pour flush)	Latrina tradicional sem laje
What type of latrine did you use outside of your compound?	Que tipo de latrina usa fora do seu composto?	WSUP buit latrine	Latrina construída pela WSUP
		Pour flush to underground pit/tank	Sistema com água corrente ligado a fossa séptica
		Pour flush to sewer system	Sistema com água corrente ligado ao esgoto
		Pour flush to elsewhere (above ground, open)	Sistema com água corrente para o local ou na superfície
		Pit latrine with concrete slab (not pour flush)	Latrina com laje de concreto (sistema sem descarga)

		Pit latrine without concrete slab (not pour flush)	Latrina tradicional sem laje
Was the latrine outside of the compound private, shared, or public?	A latrina fora do composto era privada, partilhada, ou pública	Private (in a private home or business)	Privada (Em uma casa ou estabelecimento)
		Shared (shared by a known group of people)	Partilhada (Partilhada por um grupo de pessoas conhecidas)
		Public (used by a large group of unknown people)	Público (Usada por um grupo grande de pessoas desconhecidas)
During the last 2 days, what was the primary place of defecation for all other household members older than 5 years?	Durante os últimos 2 dias, qual foi o principal local de defecação de todos os membros do agregado maires de 5 anos?	Private latrine in my house	Latrina privada dentro de casa
		Latrine in my compound (WSUP latrine)	Latrina no composto (latrina construída pela WSUP)
		Latrine in my compound (not WSUP latrine)	Latrina dentro do composto (não construída pela WSUP)
		Latrine outside of my compound	Latrina fora do composto

		Into bag or bucket	Plástico ou penico/balde
		Open defecation in the compound	Fecalismo ao céu aberto no composto
		Open defecation outside of the compound	Fecalismo ao céu aberto fora do composto
Do you and members of your household ALWAYS use the WSUP latrine for defecation (not just when you're at home)?	Você e membros do seu agregado SEMPRE usam as latrinas da WSUP para defecarem (mesmo estando fora de casa)?	Yes, we always use it	Sim, usamos sempre
		No, sometimes we do not use it	Não, por vezes não usamos
What is the main reason you (or others in your household) do not ALWAYS use the shared compound latrine built by WSUP?	Qual é a principal razão para que o entrevistado (ou outro membro do agregado) não use a latrina construída pela WSUP?	We do use it	Nós usamos
		It is dirty/smells	É suja/mal cheirosa
		It is always busy	Está sempre ocupada
		It is broken	Está danificada

		It is not private enough	Não é privada o suficiente
		It is not safe to use	Não é seguro usar
		Prefer other latrine	Prefere outra latrina
		Water to flush is expensive	A água para descarga é cara
		The pit is full	A fossa esta cheia
		Often use facilities outside of the compound	Usam muitas vezes instalações fora do composto
		Other (please specify)	Outro (especifique)
		N/A	N/A
Do you ever use the latrine for any of the following activities?	Usa a latrina para alguma das seguintes actividades?	defecation	Defecar
		urination	Urinar
		bathing	Tomar banho

		menstrual hygiene	Higiene menstrual
		changing clothes	Mudar de roupa
How do you cleanse yourself after defecating?	Como é que se limpa depois de defecar?	Wash with water	Lava com água
		Use toilet paper	Usa papel higiénico
		Other (please specify)	Outro (especifique)
		N/A	N/A
Where do you dispose of the toilet paper after use? (Do not read options to respondent. Let respondent answer and then select correct answer.)	Onde deita o papel higiénico depois de usa-lo? (Não leia as respostas. Deixe o entrevistado responder e seleccione a opção mencionada.)	latrine pit	Na latrina
		rubbish heap	No monte de lixo
		soak pit	Na fossa
		on the compound ground	No chão/solo do composto

	Other (please specify)	Outro (especifique)
	N/A	N/A
	MM/DD/YYYY H:MM	MM/DD/YYYY H:MM

Table C5: 12- and 24-month child level surveys

Question text (Eng)	Question Text (Port)	Answer text (Eng)	Answer text (Port)
N/A	N/A	Compound survey v.1.	Compound survey v.1.
N/A	N/A	MapSanStudy	MapSanStudy
N/A	N/A	Final	Final
		Draft	Draft
N/A	N/A	unique identifier for survey	unique identifier for survey
N/A	N/A	MM/DD/YYYY H:MM	MM/DD/YYYY H:MM
N/A	N/A	MM/DD/YYYY H:MM	MM/DD/YYYY H:MM
What is the compound ID?	N/A	####	####
What is the household ID?	N/A	##	##

What is the child's common name?	Qual é o nome comum da criança?		
		MM/DD/YYYY H:MM	MM/DD/YYYY H:MM
What is the child's sex?	Qual é o sexo da criança?	Female	Feminino
		Male	Masculino
Was the child previously enrolled in the study?	Esta criança fez parte do estudo de base?	Yes	Sim
		No	Não
Enter the child ID listed at baseline	Registe o ID atribuído no estudo de base	##	##
Assign the child a child ID number. (Assign the child an ID number. Since the child was not previously enrolled in the study, assign child Ids starting at 50 and going up sequentially for each new child enrolled in that HH.) (QUESTION TEXT FOR NEWLY ENROLLED CHILDREN)	Atribua um número ID a criança. (Atribua um número ID a criança. Caso a criança não tenha feito parte do estudo de base, atribua um ID iniciando pelo número 50 de maneira crescente para cada criança dentro do composto ao agregado.) (QUESTION TEXT FOR NEWLY ENROLLED CHILDREN)		
Is the child currently living in the household?	Esta criança vive actualmente no agregado?	Yes	Sim
		No	Não

Why doesn't the child live in your household any longer?	Por que é que a criança não reside mais no seu agregado?	He/she moved (stop here)	Ele/ela mudou-se (pare aqui)
		He/she died (stop here)	Ele/ela faleceu (pare aqui)
		Other (please specify)	Outro (registe a resposta do entrevistado)
		N/A	N/A
Did the child move to another house in this compound or bairro?	A criança mudou-se para uma outra casa neste composto?	Yes	Sim
		No	Não
		N/A	N/A
Is the respondent the child's mother? (If possible, the respondent should be the child's mother or female caregiver)	A entrevistada é mãe da criança? (Se possível, entrevistada deve ser a mãe ou cuidadora da criança)	Yes	Sim
		No	Não
What is the respondent's relationship to the child?	Qual é a relação do(a) entrevistado(a) com a criança?	Father	Pai

		Female relative	Familiar mulher
		Male relative	Familiar homem
		other female caregiver	Outra mulher cuidadora
		other male caregiver	Outro homem cuidador
What education did the caregiver of the child receive? (If there are several caregivers, record the highest education level.)	Qual é o nível de escolaridade do cuidador da criança? (Se existir mais de um cuidador, registe o nível de escolaridade mais elevado)	None	Nenhum
		Some primary	Ensino primário incompleto
		primary completed	Ensino primário completo
		some secondary	Ensino secundário incompleto
		secondary completed	Ensino secundário completo
		some technical training	Ensino técnico incompleto

		technical training completed	Ensino técnico completo
		some higher education	Ensino superior incompleto
		higher education completed	Ensino superior completo
		Prefer not to answer	Prefere não responder
		TRUE	TRUE
Is the child's birth certificate and vaccination card available? If available, ask the respondent to see them.	A certidão de nascimento e/ ou de vacinação da criança esta disponível? Se disponível, peça ao entrevistado para mostrar-lhe	Yes, birth certificate and vaccination card available.	Sim, certidão de nascimento e vacinação disponível.
		Only birth certificate available	Somente certidão de nascimento disponível
		Only vaccination card available	Somente cartão de vacinação disponível
		Neither available	Nenhum disponível

What is the child's birthdate? (Ask to see the chid's birth certificate or vaccination card and verify the birthdate. Once you type the date into the survey, show it to the respondent for confirmation.)	Qual é a data de nascimento da criança? (Peça ao entrevistado para mostrar-lhe a certidão de nascimento da criança ou o cartão de vacinação e verifique a data de nascimento da criança. Assim que inserir a data no questionário, mostre ao entrevistado para que este confirme.)	MM/DD/YYYY	MM/DD/YYYY
		TRUE	TRUE
If birthdate is not available, ask the respondent for the month and year of the child's birth.	Se a data de nascimento não estiver disponível, pergunte ao entrevistado pelo mês e ano de nascimento	MM/YYYY	MM/YYYY
		TRUE	TRUE
If the month and year is not available, ask for the year of the child's birth.	Se o mês não for sabido, pergunte ao entrevistado pelo ano de nascimento	YYYY	YYYY
		TRUE	TRUE
What type of facility was the child born in?	Em que tipo de unidade sanitária a criança nasceu?	Posto de saude	Posto de saúde
		Centro de saude	Centro de saúde
		hospital	Hospital distrital

		distrital	
		hospital provincial	Hospital provincial
		hospital geral	Hospital geral
		hospital central	Hospital central
		TRUE	TRUE
What is the name of the facility/hospital the child was born in? (Enter the information provided on the birth certificate, e.g. Facility name)	Qual é o nome da unidade sanitária onde a criança nasceu? (Insira a informação disponível na certidão de nascimento, ex. Nome da unidade sanitária/hospital ou local de nascimento)	facility name	facility name
		TRUE	TRUE
		TRUE	TRUE
In what province was the child born?	Em que província a criança nasceu?	Maputo Cidade	Maputo Cidade
		Maputo Província	Maputo Província
		Gaza	Gaza
		Inhambane	Inhambane
		Manica	Manica
		Sofala	Sofala

		Zambézia	Zambézia
		Tete	Tete
		Nampula	Nampula
		Cabo Delgado	Cabo Delgado
		Niassa	Niassa
		Other (please specify)	Outro (registe a resposta do entrevistado)
		N/A	N/A
Did this child receive a rotavirus vaccination? (Enumerator should check the vaccination card if available.)	Esta criança recebeu a vacinação contra o rotavirus? (Inquiridor deve verificar o cartão de vacinação se disponível.)	Yes	Sim
		No	Não
		TRUE	TRUE
What was the date of the child's first rotavirus vaccination? (Check the child's vaccination card)	Qual é a data da primeira vacinação contra o rotavirus? (Verifique o cartão de vacinação da criança)	MM/DD/YYYY	MM/DD/YYYY
		TRUE	TRUE

What was the month and year of the child's first rotavirus vaccination?	Qual foi o mês e ano da primeira vacinação contra rotavirus?	MM/YYYY	MM/YYYY
		TRUE	TRUE
What was the year of the child's first rotavirus vaccination?	Qual foi o ano da primeira vacinação contra rotavirus?	YYYY	YYYY
		TRUE	TRUE
In what type of facility did the child receive the first rotavirus vaccine?	Em que tipo de unidade sanitária a criança receber a primeira vacina contra o rotavírus ?	Posto de saude	Posto de saúde
		Centro de saude	Centro de saúde
		hospital distrital	Hospital distrital
		hospital provincial	Hospital provincial
		hospital geral	Hospital geral

		hospital central	Hospital central
		Other (please specify)	Outro (registe a resposta do entrevistado)
		N/A	N/A
		TRUE	TRUE
What is the name of the facility in which the child received the first rotavirus vaccine? (Enter the information provided on the vaccination card, e.g. Facility name and location)	Qual é o nome da unidade sanitária onde a criança recebeu a primeira vacinação contra rotavirus (Insira a informação disponível no cartão de vacinação, ex. Nome da unidade sanitária e localização.)	facility name	facility name
		TRUE	TRUE
In what province did the child receive the first rotavirus vaccination?	Em que província a criança recebeu a vacinação contra rotavirus?	Maputo Cidade	Maputo Cidade
		Maputo Província	Maputo Província

		Gaza	Gaza
		Inhambane	Inhambane
		Manica	Manica
		Sofala	Sofala
		Zambézia	Zambézia
		Tete	Tete
		Nampula	Nampula
		Cabo Delgado	Cabo Delgado
		Niassa	Niassa
		TRUE	TRUE
		TRUE	TRUE
What was the date of the child's second rotavirus vaccination?	Qual foi a data da segunda vacinação contra o rotavirus?	MM/DD/YYYY	MM/DD/YYYY

		TRUE	TRUE
What was the month and year of the child's second rotavirus vaccination?	Qual foi o mês e ano da segunda vacinação contra rotavirus?	MM/YYYY	MM/YYYY
		TRUE	TRUE
What was the year of the child's second rotavirus vaccination?	Qual foi o ano da segunda vacinação contra rotavirus?	YYYY	YYYY
		TRUE	TRUE
In what type of facility did the child receive the second rotavirus vaccine?	Em que tipo de unidade sanitária a criança receber a segunda vacina contra o rotavírus ?	Posto de saude	Posto de saúde
		Centro de saude	Centro de saúde
		hospital distrital	Hospital distrital

		hospital provincial	Hospital provincial
		hospital geral	Hospital geral
		hospital central	Hospital central
		Other (please specify)	Outro (registe a resposta do entrevistado)
		N/A	N/A
		TRUE	TRUE
What is the name of the facility in which the child received the second rotavirus vaccine?	Qual é o nome da unidade sanitária onde a criança recebeu a primeira vacinação contra rotavirus?	facility name	facility name
		TRUE	TRUE
In what province did the child receive the second rotavirus vaccination?	Em que província a criança recebeu a vacinação contra rotavirus?	Maputo Cidade	Maputo Cidade

	Maputo Província	Maputo Província
	Gaza	Gaza
	Inhambane	Inhambane
	Manica	Manica
	Sofala	Sofala
	Zambézia	Zambézia
	Tete	Tete
	Nampula	Nampula
	Cabo Delgado	Cabo Delgado
	Niassa	Niassa

Is the child currently breastfeeding?	A criança está em estágio de amamentação?	Yes, exclusively	Sim, exclusivamente.
		Yes, in addition to other foods and liquids	Sim, além de outros alimentos e líquidos.
		No	Não
		TRUE	TRUE
About how long ago did the child start eating and drinking other foods and liquids?	A cerca de quanto tempo a criança começou a comer e beber outras líquidos e alimentos?	within the past week (0-7 days)	Dentro da semana passada (0 -7 dias)
		within the past month (8-30 days)	Dentro do mês passado (8-30 dias)
		within the past 3 months (31-90 days)	Dentro dos últimos 3 meses (31-90 dias)
		within the last 6 months (91-180 days)	Dentro dos últimos 6 meses (91-180 dias)
		longer than 6 months ago (180+ days)	A mais de 6 meses (180+ dias)

Where does the child normally defecate? (Do not read answers. Let the respondent reply and then select the correct answer.)	Onde a criança normalmente defeca? (Não leia as respostas. Deixe o entrevistado responder e depois insira a resposta dada.)	In a diaper	Na fralda
		on the ground	No chão
		Potty	No penico
		In the latrine	Na latrina
		in a bag	No plástico
		Other (please specify)	Outro (registe a resposta do entrevistado)
		N/A	N/A
Where is the diaper disposed of when the child is changed?	Onde é deitada a fralda usada pela criança?	Latrine	Latrina
		Rubbish pile	Monte de lixo
		soak pit	Fossa
		it is washed	É lavada (fralda de pano)
		Other (please specify)	Outro (registe a resposta do entrevistado)

		N/A	N/A
		TRUE	TRUE
After the child has defecated on the ground, what is done with the feces?	Depois da criança defecar no chão, o que é feito com as fezes?	It is left on the ground	São deixadas no chão
		It is put into the latrine	São descartadas na latrina
		It is put into a rubbish heap	São descartadas no monte de lixo
		It is put into a soak pit	São descartadas na fossa
		Other (please specify)	Outro (registe a resposta do entrevistado)
		N/A	N/A
		TRUE	TRUE

After the child has defecated into the potty, how are the feces disposed of?	Depois da criança defecar no penico, como são deitadas as fezes?	It is emptied into the latrine	É esvaziado dentro da latrina
		It is emptied into a soak pit	É esvaziado na fossa
		It is emptied into the rubbish heap	É descartado no monte de lixo
		It is emptied onto the compound ground	Enterrada no composto
		Other (please specify)	Outro (registe a resposta do entrevistado)
		N/A	N/A
		TRUE	TRUE

After the child has defecated into the bag, how is the bag disposed of?	Depois da criança defecar no plástico, como é deitado o plástico?	Rubbish heap inside the compound	Monte do lixo dentro do composto
		Rubbish heap outside of the compound	Monte de lixo fora do composto
		soak pit	Fossa
		latrine	latrina
		Other (please specify)	Outro (registe a resposta do entrevistado)
		N/A	N/A
		TRUE	TRUE
Did this child have diarrhea at any time in the last 7 days? (Diarrhea is considered to be the passage of 3 or more loose or liquid stools per day (or more frequent passage than normal) or any stool with blood)	Esta criança teve diarreia em algum momento nos últimos 7 dias? (Considera-se diarreia a ocorrência de 3 ou mais fezes líquidas durante um dia (ou ocorrência além do normal) ou quaisquer fezes acompanhada de sangue)	Yes	Sim

		No	Não
		TRUE	TRUE
On the worst day of this episode, how many bouts of diarrhea did this child have?	No pior dia do episódio, quantas vezes a criança fez diarreia?	##	##
		TRUE	TRUE
How many days did the child have 3 or more bouts of diarrhea?	Se mais de 3 vezes, por quantos dias a criança fez diarreia 3 ou mais vezes por dia?	##	##
During this time, did the child refuse to eat on one or more days?	Durante este período, a criança recusou-se a comer em um ou mais dias?	Yes	Sim
		No	Não
		TRUE	TRUE
For how many days did the child refuse to eat?	Por quantos dias a criança recusou-se a comer?	##	##

		TRUE	TRUE
During this time, did the child have a fever on one or more days?	Durante este período, a criança teve febres em um ou mais dias?	Yes	Sim
		No	Não
		TRUE	TRUE
For how many days did the child have a fever?	Por quantos dias a criança teve febres?	##	##
		TRUE	TRUE
During this time did the child ever have blood in the stool?	Durante este período, as fezes da criança vinham acompanhadas de sangue?	Yes	Sim
		No	Não
		TRUE	TRUE

Did you ever seek treatment or advice for the diarrhea?	Procurou conselho ou tratamento para a diarreia?	Yes	Sim
		No	Não
		TRUE	TRUE
How many days after the illness began did you first seek treatment?	Quantos dias depois do início da doença procurou por conselho ou tratamento?	The same day	No mesmo dia
		The following day	No dia seguinte
		After 3 or more days	Depois de 3 ou mais dias
		TRUE	TRUE
In the last 7 days, did this child vomit on one or more days?	Nos últimos 7 dias, a criança vomitou em um ou mais dias?	Yes	Sim
		No	Não
		TRUE	TRUE

On how many days did the child vomit?	Por quantos dias a criança vomitou?	##	##
		TRUE	TRUE
In the last week, did the child have abdominal pain?	Na última semana, esta criança teve dores abdominais?	Yes	Sim
		No	Não
		TRUE	TRUE
On how many days did the child have pain?	Por quantos dias a criança queixou-se da dor?	##	##
		TRUE	TRUE
During this time, did the child refuse to eat on one or more days because of the abdominal pain?	Durante esta altura, a criança recusou-se a comer em um ou mais dias por causa de dores abdominais?	Yes	Sim
		No	Não

		TRUE	TRUE
On how many days did the child refuse to eat because of the pain?	Por quantos dias a criança recusou-se a comer por causa da dor?	##	##
		TRUE	TRUE
Was the pain in a particular place of the belly or everywhere?	A dor era em uma área específica da barriga ou em todo lado?	local	local
		everywhere	Todo o lado
		TRUE	TRUE
Please confirm whether this child was dewormed by a member of our study team following the baseline visit.	Pode por favor confirmar, se esta criança foi desparasitada por um membro do nosso estudo depois da visita do estudo de base?	Confirmed	Sim, confirmado
		Unconfirmed	Não, não confirmado
		TRUE	TRUE

Was the child dewormed by someone outside of our study team since we last visited?	Esta criança foi desparasitada por alguém fora do nosso estudo desde a nossa última visita?	Yes, by someone from MISAU	Sim, por alguém do MISAU
		Yes, by someone from a different organizatio n	Sim, por alguém de uma organização diferente
		No	Não
		TRUE	TRUE
About how long ago was the child dewormed?	A quanto tempo a criança foi desparasitada?	Within the past week (0-7 days)	Dentro da semana passada (0 -7 dias)
		Within the past month (8-30 days)	Dentro do mês passado (8-30 dias)
		within the past 3 months (31 - 90 days)	Dentro dos últimos 3 meses (31-90 dias)

		within the past 6 months (91 - 180 days)	Dentro dos últimos 6 meses (91-180 dias)
		More than 6 months ago (180+ days)	A mais de 6 meses (180+ dias)
OBSERVE: is the child wearing shoes?	OBSERVE: a criança calçava sapatos no momento da entrevista?	Yes	Sim
		No	Não
Measure the child's weight. (For children less than 24 months, use tared weight. For children older than 24 months, measure child weight alone.)	Registe o peso em Kg (Para crianças com menos de 24 meses, meça-as acompanhadas. Para crianças> 24 meses, meça-as sozinhas.)	##	##
		N/A	N/A
Measure the child's height or length in cm (For children less than 24 months, measure recumbent length. For children older than 24 months, measure standing height.)	Meça a altura ou comprimento em cm (Para crianças menores de 24 meses, use a esteira de medição. Para as crianças maiores de 24 meses, use o altimetro.)	##	##
		N/A	N/A
Is the child currently less than 5 years of age and living in the household?	Esta criança tem actualmente menos de 5 anos e vive no agregado?	Yes	Sim
		No. Stop here. Move	Não termine aqui e passe para a

		onto next child in household.	próxima criança
Why wasn't the child previously enrolled in the study?	Por que é que a criança não fez parte do estudo de base?	Born after baseline enrollment	Nascida depois da última visita da equipa do Mapsan
		Moved into compound after baseline enrollment	Mudou-se para o composto depois da última visita da equipa do Mapsan
		Refused enrollment at baseline	Recusou fazer parte do estudo anterior
		Other (please specify)	Outro (registe a resposta do entrevistado)
		N/A	N/A
How long ago did the child move into the household?	N/A	Within the past week (0-7 days)	Dentro da semana passada (0 -7 dias)
		Within the past month (8-30 days)	Dentro do mês passado (8-30 dias)
		Within the past 3	N/A

	months (31-90 days)	
	Within the past 6 months (91 - 180 days)	N/A
	More than 6 months ago (180+ days)	A mais de 6 meses (180+ dias)

APPENDIX D

BALANCE ASSESSMENT FOLLOWING LOSSES TO FOLLOW-UP

Table D1: Baseline characteristics of children available at 12-month visit and children lost to follow-up by 12-month visit pooled across all children and stratified by study arm.

	All children – lost to follow-up by 12-month		All children - available at 12-month			Intervention – lost to follow-up by 12-month		Intervention – available at 12-month			Control – lost to follow-up by 12-month		Control – available at 12-month		
	total n	n (%) | Mean (SD)	total n	n (%) | Mean (SD)	p^	total n	n (%) | Mean (SD)	total n	n (%) | Mean (SD)	p	total n	n (%) | Mean (SD)	total n	n (%) | Mean (SD)	p
Baseline outcome measures															
Primary outcome	268	229 (85%)	489	408 (83%)	0.47	111	93 (84%)	251	202 (80%)	0.46	157	136 (87%)	238	206 (87%)	0.98
Bacterial	268	209 (78%)	489	368 (75%)	0.40	111	84 (76%)	251	182 (73%)	0.53	157	125 (80%)	238	186 (78%)	0.73
Shigella	268	131 (49%)	489	201 (41%)	0.04	111	54 (49%)	251	98 (39%)	0.09	157	77 (49%)	238	103 (43%)	0.26
ETEC	268	80 (30%)	489	147 (30%)	0.95	111	32 (29%)	251	77 (31%)	0.72	157	48 (31%)	238	70 (29%)	0.81
Salmonella	268	52 (19%)	489	104 (21%)	0.54	111	21 (19%)	251	45 (18%)	0.82	157	31 (20%)	238	59 (25%)	0.24

Table D1 (continued).

Campylobacter	268	24 (9%)	489	37 (7.6%)	0.50	111	7 (6.3%)	251	14 (5.6%)	0.79	157	17 (11%)	238	23 (9.7%)	0.71
C. difficile	268	12 (4.5%)	489	23 (4.7%)	0.89	111	5 (4.5%)	251	8 (3.2%)	1.00	157	7 (4.5%)	238	15 (6.3%)	0.43
STEC	268	6 (2.2%)	489	7 (1.4%)	0.41	111	4 (3.6%)	251	5 (2%)	0.47	157	2 (1.3%)	238	2 (0.84%)	0.65
E. coli O157	268	12 (4.5%)	489	19 (3.9%)	0.69	111	8 (7.2%)	251	10 (4%)	0.19	157	4 (2.6%)	238	9 (3.8%)	0.50
Yersinia	268	ND	489	1 (0.2%)	1.00	111	ND	251	1 (0.4%)	1.00	157	ND	238	ND	-♦
Protozoan	268	142 (53%)	489	258 (53%)	0.95	111	64 (58%)	251	130 (52%)	0.30	157	78 (50%)	238	128 (54%)	0.43
Giardia	268	139 (52%)	489	248 (51%)	0.76	111	61 (55%)	251	124 (49%)	0.33	157	78 (50%)	238	124 (52%)	0.64
Cryptosporidium	268	4 (1.5%)	489	20 (4.1%)	0.05	111	3 (2.7%)	251	13 (5.2%)	0.41	157	1 (0.64%)	238	7 (2.9%)	0.15
E. histolytica	268	2 (0.75%)	489	2 (0.41%)	0.62	111	2 (1.8%)	251	2 (0.8%)	0.59	157	ND	238	ND	-♦
Viral	268	39 (15%)	489	67 (14%)	0.75	111	17 (15%)	251	35 (14%)	0.73	157	22 (14%)	238	32 (13%)	0.87
Norovirus GI/GII	268	27 (10%)	489	51 (10%)	0.88	111	12 (11%)	251	27 (11%)	0.99	157	15 (9.6%)	238	24 (10%)	0.86
Adenovirus 40/411	268	8 (3%)	489	14 (2.9%)	0.92	111	3 (2.7%)	251	6 (2.4%)	1.00	157	5 (3.2%)	238	8 (3.4%)	1.00

Table D1 (continued).

Rotavirus A	268	5 (1.9%)	489	5 (1%)	0.34	111	3 (2.7%)	251	4 (1.6%)	0.44	157	2 (1.3%)	238	1 (0.42%)	0.57
Coinfection	268	159 (59%)	489	283 (58%)	0.70	111	65 (59%)	251	140 (56%)	0.62	157	94 (60%)	238	143 (60%)	0.97
Number of infections	268	1.9 (1.2)	489	1.8 (1.2)	0.41	111	1.9 (1.3)	251	1.7 (1.2)	0.14	157	1.8 (1.1)	238	1.9 (1.2)	0.73
Reported diarrhea	366	43 (12%)	614	83 (14%)	0.42	150	14 (9.3%)	300	45 (15%)	0.09	216	29 (13%)	314	38 (12%)	0.65
log(AAT) (mg/g)	248	-0.49 (0.53)	462	-0.45 (0.5)	0.24	105	-0.42 (0.51)	251	-0.43 (0.49)	0.89	143	-0.55 (0.54)	221	-0.46 (0.51)	0.15
log(MPO) (ng/mL)	249	3.7 (0.4)	468	3.7 (0.46)	0.95	105	3.6 (0.38)	251	3.6 (0.47)	0.99	144	3.7 (0.42)	226	3.7 (0.44)	0.72
log(NEO) (nmol/L)	229	3.0 (0.53)	439	3.0 (0.54)	0.57	94	3.0 (0.53)	251	2.9 (0.52)	0.46	135	3.0 (0.53)	214	3.0 (0.55)	0.11
log(CAL) (ng/mL)	248	5.4 (0.45)	467	5.4 (0.5)	0.97	105	5.4 (0.39)	251	5.4 (0.49)	0.82	143	5.4 (0.48)	225	5.4 (0.5)	0.86
EED score	227	5 (2.6)	435	5.1 (2.6)	0.75	94	5.1 (2.5)	251	4.9 (2.6)	0.51	133	4.9 (2.6)	210	5.2 (2.5)	0.26
Baseline demographic, socio-economic, environmental factors															
Child-level variables															
Child age, days	353	700 (410)	614	690 (410)	0.90	143	700 (420)	300	690 (410)	0.92	210	700 (390)	314	700 (410)	0.95
Child sex, female	350	174 (50%)	619	321 (52%)	0.52	142	77 (54%)	303	150 (50%)	0.35	208	97 (47%)	316	171 (54%)	0.09

Table D1 (continued).

Child is breastfed, with or without complementary feeding	366	107 (29%)	614	209 (34%)	0.12	150	45 (30%)	300	100 (33%)	0.48	216	62 (29%)	314	109 (35%)	0.15
Child is exclusively breastfed	366	36 (9.8%)	614	53 (8.6%)	0.53	150	14 (9.3%)	300	26 (8.7%)	0.82	216	22 (10%)	314	27 (8.6%)	0.54
Child feces disposal in latrine or potty	366	116 (32%)	614	173 (28%)	0.24	150	47 (31%)	300	94 (31%)	1.00	216	69 (32%)	314	79 (25%)	0.09
Child wears diapers	365	236 (65%)	614	406 (66%)	0.64	149	102 (68%)	300	194 (65%)	0.43	216	134 (62%)	314	212 (68%)	0.19
Caregiver completed primary school	366	195 (53%)	619	336 (54%)	0.76	150	71 (47%)	303	172 (57%)	0.06	216	124 (57%)	316	164 (52%)	0.21
Child's mother is alive	359	354 (99%)	595	581 (98%)	0.30	147	146 (99%)	290	282 (97%)	0.15	212	208 (98%)	305	299 (98%)	0.95
Respondent is child's mother	358	237 (66%)	610	418 (69%)	0.46	146	92 (63%)	299	192 (64%)	0.81	212	145 (68%)	311	226 (73%)	0.29
Household-level variables															
Household population	370	5.7 (2.6)	620	6.4 (3.3)	0.00	152	6.3 (3.1)	251	6.9 (3.5)	0.10	218	5.2 (2.1)	317	6 (3)	0.00
Household wealth score, 1 (poorer) - 100 (wealthier)†	369	0.44 (0.099)	620	0.43 (0.1)	0.18	152	0.43 (0.1)	251	0.43 (0.1)	1.00	217	0.45 (0.096)	317	0.44 (0.1)	0.11

Table D1 (continued).

Household crowding, >3 persons/room	369	46 (12%)	620	122 (20%)	0.00	152	24 (16%)	303	67 (22%)	0.11	217	22 (10%)	317	55 (17%)	0.02
Household floor is covered‡	369	350 (95%)	620	580 (94%)	0.40	152	139 (91%)	303	276 (91%)	0.90	217	211 (97%)	317	304 (96%)	0.41
Household wall made of bricks, concrete, or similar‡	369	244 (66%)	620	403 (65%)	0.72	152	91 (60%)	303	185 (61%)	0.81	217	153 (71%)	317	218 (69%)	0.67
Household drinking water source inside compound	366	287 (78%)	610	470 (77%)	0.62	151	124 (82%)	299	244 (82%)	0.89	215	163 (76%)	311	226 (73%)	0.42
Latrine used by household															
Has a ceramic or masonry pedestal‡	365	135 (37%)	606	226 (37%)	0.92	151	55 (36%)	298	124 (42%)	0.29	214	80 (37%)	308	102 (33%)	0.31
Has a drophole cover‡	366	195 (53%)	608	362 (60%)	0.06	151	86 (57%)	298	193 (65%)	0.11	215	109 (51%)	310	169 (55%)	0.39
Compound-level variables															
Compound population	373	19 (14)	620	21 (15)	0.01	153	24 (20)	251	26 (18)	0.27	220	15 (6.1)	317	17 (8)	0.02
Number of households	372	4.7 (4.4)	620	5.2 (4.5)	0.09	153	6 (6.4)	251	6.1 (5.6)	0.82	219	3.8 (1.8)	317	4.4 (2.9)	0.02

Table D1 (continued).

Number of water taps inside the compound	364	1.2 (1)	609	1.5 (2.2)	0.00	149	1.4 (1.2)	251	2.1 (2.8)	0.01	215	0.97 (0.83)	310	1 (1.1)	0.66
Number of latrines in the compound	364	1.1 (0.65)	609	1.1 (0.64)	0.97	149	1.3 (0.98)	251	1.2 (0.86)	0.82	215	1 (0.2)	310	1 (0.25)	0.53
# households/latrine	369	4.1 (2.7)	606	4.5 (3.2)	0.03	150	4.5 (3.7)	251	4.9 (4)	0.36	219	3.8 (1.8)	312	4.1 (2.1)	0.03
Latrine walls made of brick, concrete or similar‡	364	111 (30%)	610	194 (32%)	0.67	149	53 (36%)	300	110 (37%)	0.82	215	58 (27%)	310	84 (27%)	0.98
Compound population density, persons/square meter*	359	0.078 (0.045)	612	0.084 (0.046)	0.07	150	0.089 (0.05)	230	0.092 (0.051)	0.58	209	0.07 (0.039)	313	0.076 (0.039)	0.12
Compound has electricity that normally functions	373	332 (89%)	620	560 (90%)	0.51	153	137 (90%)	303	286 (94%)	0.06	220	195 (89%)	317	274 (86%)	0.45
Compound prone to flooding	373	227 (61%)	620	382 (62%)	0.81	153	90 (59%)	303	168 (55%)	0.49	220	137 (62%)	317	214 (68%)	0.21
Any animals observed in compound‡	373	227 (61%)	620	399 (64%)	0.27	153	98 (64%)	303	208 (69%)	0.32	220	129 (59%)	317	191 (60%)	0.71

Table D1 (continued).

Dog observed‡	373	23 (6.2%)	620	53 (8.6%)	0.17	153	14 (9.2%)	303	34 (11%)	0.50	220	9 (4.1%)	317	19 (6%)	0.33
Chicken or duck(s) observed‡	373	35 (9.4%)	620	96 (15%)	0.01	153	8 (5.2%)	303	51 (17%)	0.00	220	27 (12%)	317	45 (14%)	0.52
Cats observed‡	373	206 (55%)	620	344 (55%)	0.94	153	86 (56%)	303	175 (58%)	0.75	220	120 (55%)	317	169 (53%)	0.78

Data are n (%) or mean (SD) and collected by questionnaire unless otherwise noted. ^ p-values calculated using t tests or chi-square tests (Fisher's exact used in place of chi-square test when cell counts <5). ND = not detected. ♦ p-value could not be calculated due to non-detects [†]Assessed using Simple Poverty Scorecard for Mozambique, [‡]Data collected by direct observation, [*]Calculated as # of people living in the compound divided by the area of the compound in square meters. Area measurement described previously (Chapter 2).

Table D2: Baseline characteristics of children available at 24-month visit and children lost to follow-up by 24-month visit

	All children – lost to follow-up		All children - available at 24-month			Intervention – lost to follow-up		Intervention – available at 24-month			Control – lost to follow-up		Control – available at 24-month		
	total n	n (%) | Mean (SD)	total n	n (%) | Mean (SD)	p^	total n	n (%) | Mean (SD)	total n	n (%) | Mean (SD)	p	total n	n (%) | Mean (SD)	total n	n (%) | Mean (SD)	p
Baseline outcome measures															
Primary outcome	361	302 (84%)	396	335 (85%)	0.72	152	122 (80%)	210	173 (82%)	0.61	209	180 (86%)	186	162 (87%)	0.78
Bacterial	361	276 (76%)	396	301 (76%)	0.89	152	111 (73%)	210	155 (74%)	0.87	209	165 (79%)	186	146 (78%)	0.91
Shigella	361	174 (48%)	396	158 (40%)	0.02	152	70 (46%)	210	82 (39%)	0.18	209	104 (50%)	186	76 (41%)	0.08
ETEC	361	114 (32%)	396	113 (29%)	0.36	152	49 (32%)	210	60 (29%)	0.45	209	65 (31%)	186	53 (28%)	0.57
Salmonella	361	69 (19%)	396	87 (22%)	0.33	152	28 (18%)	210	38 (18%)	0.94	209	41 (20%)	186	49 (26%)	0.11
Campylobacter	361	31 (8.6%)	396	30 (7.6%)	0.61	152	9 (5.9%)	210	12 (5.7%)	0.93	209	22 (11%)	186	18 (9.7%)	0.78
C. difficile	361	17 (4.7%)	396	18 (4.6%)	0.92	152	5 (3.3%)	210	8 (3.8%)	0.55	209	12 (5.7%)	186	10 (5.4%)	0.87
STEC	361	8 (2.2%)	396	5 (1.3%)	0.40	152	6 (4%)	210	3 (1.4%)	0.47	209	2 (0.96 %)	186	2 (1.1%)	0.65
E. coli O157	361	14 (3.9%)	396	17 (4.3%)	0.77	152	8 (5.3%)	210	10 (4.8%)	0.83	209	6 (2.9%)	186	7 (3.8%)	0.62

Table D2 (continued).

Yersinia	361	1 (0.28 %)	396	ND	1.00	152	1 (0.66 %)	210	ND	1.00	209	ND	186	ND	-♦
Protozoan	361	186 (52%)	396	214 (54%)	0.49	152	78 (51%)	210	116 (55%)	0.46	209	108 (52%)	186	98 (53%)	0.84
Giardia	361	183 (51%)	396	204 (52%)	0.82	152	75 (49%)	210	110 (52%)	0.57	209	108 (52%)	186	94 (51%)	0.82
Cryptosporidium	361	4 (1.1%)	396	20 (5.1%)	0.05	152	3 (2%)	210	13 (6.2%)	0.41	209	1 (0.48 %)	186	7 (3.8%)	0.15
E. histolytica	361	2 (0.55 %)	396	2 (0.51 %)	0.62	152	2 (1.3%)	210	2 (0.95%)	0.59	209	ND	186	ND	-♦
Viral	361	50 (14%)	396	56 (14%)	0.91	152	19 (13%)	210	33 (16%)	0.39	209	31 (15%)	186	23 (12%)	0.48
Norovirus GI/GII	361	35 (9.7%)	396	43 (11%)	0.60	152	12 (7.9%)	210	27 (13%)	0.13	209	23 (11%)	186	16 (8.6%)	0.42
Adenovirus 40/411	361	8 (2.2%)	396	14 (3.5%)	0.28	152	2 (1.3%)	210	7 (3.3%)	1.00	209	6 (2.9%)	186	7 (3.8%)	0.62
Rotavirus A	361	7 (1.9%)	396	3 (0.76 %)	0.34	152	5 (3.3%)	210	2 (0.95%)	0.44	209	2 (0.96 %)	186	1 (0.54%)	0.57
Coinfection	361	210 (58%)	396	232 (59%)	0.91	152	85 (56%)	210	120 (57%)	0.82	209	125 (60%)	186	112 (60%)	0.93
Number of infections	361	1.8 (1.2)	396	1.8 (1.2)	0.61	152	1.8 (1.3)	210	1.8 (1.2)	0.83	209	1.9 (1.2)	186	1.8 (1.1)	0.69
Reported diarrhea	472	51 (11%)	508	75 (15%)	0.06	189	19 (10%)	261	40 (15%)	0.10	283	32 (11%)	247	35 (14%)	0.32

Table D2 (continued).

log(AAT) (mg/g)	335	-0.47 (0.51)	375	-0.45 (0.51)	0.60	143	-0.41 (0.5)	210	-0.43 (0.49)	0.72	192	-0.52 (0.52)	172	-0.47 (0.53)	0.45
log(MPO) (ng/mL)	338	3.7 (0.42)	379	3.6 (0.45)	0.34	143	3.7 (0.42)	210	3.6 (0.46)	0.24	195	3.7 (0.43)	175	3.7 (0.44)	0.86
log(NEO) (nmol/L)	319	3 (0.56)	349	3 (0.52)	0.47	134	2.9 (0.55)	210	2.9 (0.51)	0.91	185	3 (0.57)	164	3.1 (0.52)	0.19
log(CAL) (ng/mL)	336	5.5 (0.45)	379	5.4 (0.5)	0.15	143	5.5 (0.43)	210	5.4 (0.49)	0.12	193	5.5 (0.47)	175	5.4 (0.52)	0.64
EED score	316	5 (2.6)	346	5 (2.5)	0.98	134	5.1 (2.6)	210	4.8 (2.5)	0.40	182	5 (2.6)	161	5.3 (2.6)	0.37
Baseline demographic, socio-economic, environmental factors															
Child-level variables															
Child age, days	460	690 (410)	507	700 (410)	0.73	183	680 (420)	261	700 (410)	0.47	277	700 (400)	247	690 (410)	0.87
Child sex, female	463	234 (51%)	506	261 (52%)	0.75	182	91 (50%)	263	136 (52%)	0.72	281	143 (51%)	243	125 (51%)	0.90
Child is breastfed, with or without complementary feeding	472	142 (30%)	508	174 (34%)	0.16	189	59 (31%)	261	86 (33%)	0.70	283	83 (29%)	247	88 (36%)	0.12
Child is exclusively breastfed	472	52 (11%)	508	37 (7.3%)	0.04	189	22 (12%)	261	18 (6.9%)	0.08	283	30 (11%)	247	19 (7.7%)	0.25
Child feces disposal in latrine or potty	472	150 (32%)	508	139 (27%)	0.13	189	59 (31%)	261	82 (31%)	0.96	283	91 (32%)	247	57 (23%)	0.02

Table D2 (continued).

Child wears diapers	471	298 (63%)	508	344 (68%)	0.14	188	124 (66%)	261	172 (66%)	0.99	283	174 (61%)	247	172 (70%)	0.05
Caregiver completed primary school	474	256 (54%)	511	275 (54%)	0.95	190	99 (52%)	263	144 (55%)	0.58	284	157 (55%)	248	131 (53%)	0.57
Child's mother is alive	464	457 (98%)	490	478 (98%)	0.30	186	185 (99%)	251	243 (97%)	0.05	278	272 (98%)	239	235 (98%)	0.69
Respondent is child's mother	464	316 (68%)	504	339 (67%)	0.78	185	120 (65%)	260	164 (63%)	0.70	279	196 (70%)	244	175 (72%)	0.71
Household-level variables															
Household population	479	5.6 (2.6)	511	6.7 (3.4)	0.00	192	6.1 (3)	Percent	7.1 (3.6)	0.00	287	5.2 (2.2)	248	6.3 (3)	0.00
Household wealth score, 1 (poorer) - 100 (wealthier)†	478	0.44 (0.097)	511	0.43 (0.11)	0.09	192	0.43 (0.1)	210	0.42 (0.11)	0.28	286	0.45 (0.095)	248	0.44 (0.1)	0.34
Household crowding, >3 persons/room	478	54 (11%)	511	114 (22%)	0.00	192	22 (11%)	263	69 (26%)	0.00	286	32 (11%)	248	45 (18%)	0.02
Household floor is covered‡	478	457 (96%)	511	473 (93%)	0.04	192	178 (93%)	263	237 (90%)	0.33	286	279 (98%)	248	236 (95%)	0.14
Household wall made of bricks, concrete, or similar‡	478	306 (64%)	511	341 (67%)	0.37	192	121 (63%)	263	155 (59%)	0.38	286	185 (65%)	248	186 (75%)	0.01
Household drinking water source inside compound	475	367 (77%)	501	390 (78%)	0.83	190	150 (79%)	260	218 (84%)	0.18	285	217 (76%)	241	172 (71%)	0.21

Table D2 (continued).

Latrine used by household														
Has a ceramic or masonry pedestal‡	474	175 (37%)	497	186 (37%)	0.87	190	70 (37%)	259	109 (42%)	0.26	284	105 (37%)	238	77 (32%)	0.27
Has a drophole cover‡	474	258 (54%)	500	299 (60%)	0.09	190	114 (60%)	259	165 (64%)	0.42	284	144 (51%)	241	134 (56%)	0.26
Compound-level variables														
Compound population	482	18 (14)	511	22 (15)	0.00	193	23 (19)	210	27 (18)	0.06	289	15 (6.5)	248	17 (8.1)	0.00
Number of households	481	4.7 (4.3)	511	5.3 (4.7)	0.03	193	5.9 (6.2)	210	6.1 (5.7)	0.70	288	3.9 (1.9)	248	4.4 (3.1)	0.01
Number of water taps inside the compound	468	1.2 (1.3)	505	1.1 (0.62)	0.00	186	1.5 (1.8)	210	2.2 (2.8)	0.00	282	0.98 (0.91)	243	1 (1.1)	0.69
Number of latrines in the compound	468	1.1 (0.66)	505	1.1 (0.62)	0.96	186	1.3 (1)	210	1.2 (0.82)	0.36	282	1 (0.2)	243	1 (0.25)	0.52
# households/latrine	475	4.1 (2.6)	500	4.6 (3.4)	0.00	189	4.4 (3.4)	210	5 (4.2)	0.11	286	3.8 (1.9)	245	4.2 (2.1)	0.03
Latrine walls made of brick, concrete or similar‡	469	138 (29%)	505	167 (33%)	0.22	187	63 (34%)	262	100 (38%)	0.33	282	75 (27%)	243	67 (28%)	0.80
Compound population density, persons/square meter*	469	0.079 (0.042)	502	0.084 (0.049)	0.07	192	0.085 (0.044)	191	0.096 (0.055)	0.03	277	0.075 (0.04)	245	0.072 (0.038)	0.50

Table D2 (continued).

Compound has electricity that normally functions	482	434 (90%)	511	458 (90%)	0.83	193	181 (94%)	263	242 (92%)	0.47	289	253 (88%)	248	216 (87%)	0.88
Compound prone to flooding	482	293 (61%)	511	316 (62%)	0.73	193	111 (58%)	263	147 (56%)	0.73	289	182 (63%)	248	169 (68%)	0.21
Any animals observed in compound‡	482	285 (59%)	511	341 (67%)	0.01	193	123 (64%)	263	183 (70%)	0.19	289	162 (56%)	248	158 (64%)	0.07
Dog observed‡	482	26 (5.4%)	511	50 (9.8%)	0.01	193	16 (8.3%)	263	32 (12%)	0.18	289	10 (3.5%)	248	18 (7.3%)	0.05
Chicken or duck(s) observed‡	482	59 (12%)	511	72 (14%)	0.39	193	20 (10%)	263	39 (15%)	0.16	289	39 (13%)	248	33 (13%)	0.95
Cats observed‡	482	252 (52%)	511	298 (58%)	0.06	193	108 (56%)	263	153 (58%)	0.64	289	144 (50%)	248	145 (58%)	0.05

Data are n (%) or mean (SD) and collected by questionnaire unless otherwise noted. ^ p-values calculated using t tests or chi-square tests (Fisher's exact used in place of chi-square test when cell counts <5). ND = not detected. ♦ p-value could not be calculated due to non-detects [†]Assessed using Simple Poverty Scorecard for Mozambique, [‡]Data collected by direct observation, [*]Calculated as # of people living in the compound divided by the area of the compound in square meters. Area measurement described previously (Chapter 2).

Table D3: Characteristics of children enrolled at baseline and children newly enrolled at 12-month follow-up (measurements at 12-month).

	All Children -New Enrollees By 12-Month		All Children – Enrolled At Baseline			Intervention - New Enrollees By 12-Month		Intervention – Enrolled At Baseline			Control - New Enrollees By 12-Month		Control – Enrolled At Baseline		
	tot n	n (%) | mean (sd)	tot n	n (%) | mean (sd)	p	tot n	n (%) | mean (sd)	tot n	n (%) | mean (sd)	p	tot n	n (%) | mean (sd)	tot n	n (%) | mean (sd)	p
Child-level variables															
Child Sex, Female	309	155 (50%)	619	321 (52%)	0.63	153	81 (53%)	303	150 (50%)	0.49	156	74 (47%)	316	171 (54%)	0.17
Caregiver Completed Primary School	305	143 (47%)	619	306 (49%)	0.47	151	79 (52%)	303	149 (49%)	0.53	154	64 (42%)	316	157 (50%)	0.10
Respondent is Child's Mother	302	235 (78%)	567	366 (65%)	0.00	149	112 (75%)	283	177 (63%)	0.01	153	123 (80%)	284	189 (67%)	0.00
Household-level variables															
Household Population	317	6.3 (3.3)	620	6.5 (3.2)	0.31	153	6.2 (3.2)	303	6.9 (3.3)	0.05	164	6.4 (3.5)	317	6.2 (3)	0.57

Table D3 (continued).

Household Wealth Score, 1 (Poorer) - 100 (Wealthier)†	317	0.39 (0.11)	620	0.4 (0.11)	0.64	153	0.4 (0.1)	270	0.39 (0.1)	0.34	164	0.39 (0.11)	317	0.4 (0.11)	0.14
Household Crowding, >3 Persons/Room	317	138 (44%)	620	214 (35%)	0.01	153	61 (40%)	303	103 (34%)	0.22	164	77 (47%)	317	111 (35%)	0.01
Household Floor Is Covered‡	317	301 (95%)	620	589 (95%)	0.98	153	145 (95%)	303	286 (94%)	0.87	164	156 (95%)	317	303 (96%)	0.82
Household Wall Made Of Bricks, Concrete, Or Similar‡	317	186 (59%)	620	402 (65%)	0.07	153	85 (56%)	303	189 (62%)	0.16	164	101 (62%)	317	213 (67%)	0.22
Compound-level variables															
Compound Population	320	24 (26)	620	23 (22)	0.52	155	31 (35)	270	28 (29)	0.40	165	17 (8.7)	317	18 (9.6)	0.68
Number Of Households	320	5.4 (5.6)	620	5.2 (4.7)	0.54	155	7 (7.4)	270	6.3 (5.9)	0.28	165	4 (2.2)	317	4.2 (2.9)	0.38

Table D3 (continued).

Compound Population Density, Persons/Square Meter*	314	0.084 (0.051)	612	0.085 (0.049)	0.07	153	0.09 (0.058)	207	0.091 (0.054)	0.78	161	0.078 (0.043)	313	0.08 (0.04)	0.69
Compound Has Electricity That Normally Functions	320	300 (94%)	620	580 (94%)	0.91	155	149 (96%)	303	291 (96%)	0.96	165	151 (92%)	317	289 (91%)	0.90
Compound Prone To Flooding	320	116 (36%)	620	223 (36%)	0.93	155	53 (34%)	303	89 (29%)	0.29	165	63 (38%)	317	134 (42%)	0.39
Any Animals Observed In Compound‡	320	271 (85%)	616	510 (83%)	0.46	155	139 (90%)	303	271 (89%)	0.94	165	132 (80%)	313	239 (76%)	0.36
Dog Observed‡	320	80 (25%)	616	135 (22%)	0.29	155	42 (27%)	303	75 (25%)	0.59	165	38 (23%)	313	60 (19%)	0.32
Chicken Or Duck(S) Observed‡	320	42 (13%)	616	78 (13%)	0.84	155	24 (15%)	303	43 (14%)	0.71	165	18 (11%)	313	35 (11%)	0.93
Cats	320	245	616	474	0.90	155	126	303	252	0.62	165	119	313	222	0.78

Observed‡		(77%)		(77%)			(81%)		(83%)			(72%)		(71%)	

Data are n (%) or mean (SD) and collected by questionnaire unless otherwise noted. Tot n represents denominator for given variable. p-values calculated using t tests or chi-square tests. †Assessed using Simple Poverty Scorecard for Mozambique, ‡Data collected by direct observation, *Calculated as # of people living in the compound divided by the area of the compound in square meters. Area measurement described previously (Chapter 2).

Table D4: Characteristics of children enrolled at baseline and children newly enrolled at 24-month follow-up (measurements at 24-month).

	All Children - New enrollees by 24-month		All Children – Enrolled at baseline			Intervention - New enrollees by 24-month		Intervention– Enrolled at baseline			Control - new enrollees by 24-month		Control – Enrolled at baseline		
	tot n	n (%) | Mean (SD)	tot n	n (%) | Mean (SD)	p	tot n	n (%) | Mean (SD)	tot n	n (%) | Mean (SD)	p	tot n	n (%) | Mean (SD)	tot n	n (%) | Mean (SD)	p
Child level variables															
Child sex, female	423	188 (44%)	506	261 (52%)	0.03	201	92 (46%)	263	136 (52%)	0.21	222	96 (43%)	243	125 (51%)	0.08
Caregiver completed primary school	422	163 (39%)	511	200 (39%)	0.87	201	80 (40%)	263	112 (43%)	0.55	221	83 (38%)	248	88 (35%)	0.64
Respondent is child's mother	376	295 (78%)	423	260 (61%)	0.00	182	134 (74%)	225	130 (58%)	0.00	194	161 (83%)	198	130 (66%)	0.00
Household level variables															
Household population	478	6.3 (3.4)	511	6.6 (3.1)	0.08	230	5.9 (2.8)	239	6.8 (3.1)	0.00	248	6.6 (3.8)	248	6.5 (3)	0.61
Household wealth score, 1 (poorer) - 100 (wealthier)†	467	0.41 (0.11)	511	0.41 (0.11)	0.95	225	0.42 (0.01)	239	0.41 (0.1)	0.09	242	0.4 (0.11)	248	0.42 (0.12)	0.17
Household crowding, >3 persons/room	467	109 (23%)	511	138 (27%)	0.19	225	41 (18%)	263	64 (24%)	0.10	242	68 (28%)	248	74 (30%)	0.67

Table D4 (continued).

Household floor is covered‡	467	458 (98%)	511	488 (96%)	0.02	225	221 (98%)	263	248 (94%)	0.03	242	237 (98%)	248	240 (97%)	0.43
Household wall made of bricks, concrete, or similar‡	467	297 (64%)	511	356 (70%)	0.04	225	137 (61%)	263	174 (66%)	0.23	242	160 (66%)	248	182 (73%)	0.08
Compound level variables															
Compound population	494	21 (17)	511	21 (15)	0.70	237	25 (21)	239	25 (19)	0.88	257	17 (8.9)	248	18 (9.5)	0.52
Number of households	494	5.5 (5.5)	511	5.3 (4.9)	0.46	237	6.7 (7)	239	6.2 (6.1)	0.37	257	4.4 (3.2)	248	4.3 (2.8)	0.68
Compound population density, persons/square meter*	488	0.08 (0.047)	502	0.08 (0.046)	0.07	233	0.086 (0.052)	153	0.087 (0.053)	0.85	255	0.075 (0.041)	245	0.073 (0.037)	0.60
Compound has electricity that normally functions	494	472 (96%)	511	489 (96%)	0.91	237	232 (98%)	263	256 (97%)	0.69	257	240 (93%)	248	233 (94%)	0.79
Compound prone to flooding	494	185 (37%)	511	186 (36%)	0.73	237	79 (33%)	263	94 (36%)	0.57	257	106 (41%)	248	92 (37%)	0.34

Table D4 (continued).

Any animals observed in compound‡	494	361 (73%)	511	387 (76%)	0.33	237	175 (74%)	263	224 (85%)	0.00	257	186 (72%)	248	163 (66%)	0.11
Dog observed‡	494	77 (16%)	511	72 (14%)	0.50	237	37 (16%)	263	42 (16%)	0.91	257	40 (16%)	248	30 (12%)	0.26
Chicken or duck(s) observed‡	494	52 (11%)	511	64 (13%)	0.32	237	20 (8.4%)	263	41 (16%)	0.02	257	32 (12%)	248	23 (9.3%)	0.25
Cats observed‡	494	344 (70%)	511	361 (71%)	0.73	237	166 (70%)	263	206 (78%)	0.03	257	178 (69%)	248	155 (63%)	0.11

Data are n (%) or mean (SD) and collected by questionnaire unless otherwise noted. New enrollees at 24-month includes children enrolled at 12-month. Tot n represents denominator for given variable. p-values calculated using t tests or chi-square tests. †Assessed using Simple Poverty Scorecard for Mozambique, ‡Data collected by direct observation, *Calculated as # of people living in the compound divided by the area of the compound in square meters. Area measurement described previously (Chapter 2).

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